AMPHETAMINE – INDUCED EXTINCTION DEFICITS AND BEHAVIOURAL SENSITISATION : THE ROLE OF THE VENTRAL TEGMENTAL AREA AND BASOLATERAL AMYGDALA

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Masters of Science in Psychology

at the

University of Canterbury

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by

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2005

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ACKNOWLEDGMENTS

I would like to thank my supervisor, Professor Larry Kokkinidis for all his help and guidance throughout my thesis, and my co-supervisor Associate Professor Rob Hughes for taking the time to give me advice. In particular I would like to thank my supervisor Professor Kokkinidis for his ability to challenge my ideas and also for his willingness to be challenged on academic matters. I greatly appreciate the time and expertise that both of my supervisors have shared with me. I would also like to thank all of the staff and students from the animal laboratory who have helped to make this happen. In particular, I would like to thank my fellow students, Amanda Taylor and Jeff Kellett for all their help, patience and support. Thank-you.

ABBREVIATIONS

DA	dopamine
DAT	dopamine transporter
6 – OHDA	6 – hydroxydopamine
A10	ventral tegmental area
A9	substantia nigra
ANOVA	analysis of variance
CS	conditioned stimulus
UCS	unconditioned stimulus
CR	conditioned response
VTA	ventral tegmental area
NMDA(R)	N-methyl-D-aspartate (receptor)
BLA	basolateral nucleus of the amygdala
SN	substantia nigra
NACC	nucleus accumbens
AMP	d-amphetamine
PnC	caudal pontine reticular nucleus
AP	anterior → posterior
ML	medial → lateral
DV	dorsal → ventral
ISI	inter-stimulus-interval
I.P.	intra-peritoneal
LTP	long-term potentiation
LTD	long-term depression
AMPA (R)	amino-3-hydroxy-5-methylisoxazole-4-propionic acid (receptor)
Mg^{2+}	magnesium
Ca^{2+}	calcium
Na^{+}	sodium
K^{+}	potassium

ABSTRACT

Paranoia, phobias, and other disorders involving abnormal fear, share a common dysregulation of intrusive thought. These disruptive thoughts elicit defensive behavioural responses in the absence of a genuine threat. To understand the neural basis of fear responding the present study employed an animal paradigm of abnormal fear involving the chronic administration of d-amphetamine. D-amphetamine (2.5 µg / 0.5 µl side) was infused into the ventral tegmental area (VTA) – a region whose dopaminergic projections are critical to fear neurocircuitry – prior to each of three extinction sessions. As d–amphetamine is especially effective in potentiating the release of mid–brain dopamine, and the hyperactivity of dopaminergic transmission has been repeatedly implicated in the genesis of abnormal fear, it was predicted that an infusion of the drug prior to extinction would result in an extinction deficit. The chronic administration of amphetamine to the VTA prior to extinction sessions was found to interfere with the subject's ability to extinguish a previously conditioned fear, as tested on a subsequent fear-potentiated startle test. This result was not due to chronic amphetamine administration enhancing the baseline levels of conditioned fear. Administration of amphetamine (2.5 µg / 0.5 µl side) to a terminal region of the VTA dopaminergic projections, the basolateral amygdala, did not produce the extinction deficits yielded from the VTA. This regional specificity raises interesting questions about the role of somatodendritic dopamine release in the maintenance of abnormal fear.

As an adjunct to the primary aim of this study, the effects of behavioural sensitisation to psycho-stimulants were also assessed. This helped to establish that the infusions were reaching the correct target area and additionally, the behaviourally sensitised state of the subjects further helped to align the findings of this study with the literature surrounding psycho-stimulant induced fear.

I N T R O D U C T I O N

Fear, the evolutionary survival tool, functions as an emotional motivator and a behavioural precipitator. For the last century, the neural underpinnings of this emotional state have been sought. Gradually, as the neurocircuitry involved becomes more apparent, so too do the number of questions that remain unanswered by these neurobiological studies. One of the most conspicuously unsolved areas is the neurocircuitry involved in abnormal fear. Adaptively disadvantageous, abnormal fear may quite simply be a malfunction of the neurocircuitry underlying normal fear. However, the possibility remains that rather than a simple malfunction of the known neurocircuitry, abnormal fear has distinct neural underpinnings. In a field of research still in its' infancy the quest continues to establish the neural basis of abnormal fear.

1 Emotionality and the mesolimbic system

1. 1. Fear

Fear is the emotional state which arises as part of a biological response to a threatening stimulus. Each species has an inherent repertoire of defensive behaviours that the emotion of fear will elicit. These defensive behaviours, educed by fear, serve as potent tools in the survival of a species. In mammals, behaviours that are usually associated with fear responding are, freezing, increased startle amplitude,, autonomic changes, and hypoalgesia (Rosen &

Schulkin, 1998). The quintessential characteristic of fear is that it is an adaptively advantageous emotion.

As normal fear can be characterised by its' adaptive benefits, abnormal fear can be defined in terms of its' maladaptive nature. Abnormal fear is the physiological manifestation of fear in the absence of a real or immediate threat. The arousal of the autonomic nervous system occurs without conscious control, and it remains a contentious issue whether the centralised emotional state of fear occurs prior to, concurrently with, or as a result of, this physiological arousal.

1. 2. Measurement of Fear and Fear – Potentiated Startle

To experimentally produce the centralised emotional state of fear animal models commonly employ Pavlovian conditioning. Pavlovian fear conditioning involves the prolonged pairing of a non-aversive stimulus, known as the conditioned stimulus (CS), with a naturally aversive stimulus, known as the unconditioned stimulus (UCS). Through this process the unconditioned stimulus indirectly acquires aversive properties. The subject will then display fearful behaviour when presented with the unconditioned stimulus, due to its prior associations. The conditioned fear may then be extinguished by the continuous presentation of the unconditioned stimulus without the aversive consequence. If the conditioned fear is unable to be extinguished, it is considered that the subject has acquired a maladaptive or abnormal fear of the stimulus.

One paradigm commonly used in animal studies to measure fear is known as fear-potentiated startle. Startle is a naturally occurring reflex to a fear-evoking stimulus.

If fear is viewed as a motivational state, it should augment behavioural responses that occur simultaneously with it. This hypothesis led to the investigation of the effect of a fearful state on the acoustic startle reflex (Brown, Kalish, & Farber, 1951). Acoustic startle amplitudes were found to increase relative to baseline in the presence of a fear-evoking stimulus.(Brown et al., 1951). It is the augmentation of the startle response rather than startle response itself that is the measure of fear. By measuring the augmentation of a subject's startle response, one can infer from its' potency, the level of fear the subject experiences when presented with that particular fear - evoking stimulus. The startle paradigm has considerable face validity as a behavioural manifestation of fear in animals primarily due to its' ability to be reliably produced across species . Acoustic startle has been widely used as a reliable nonverbal index of fear and anxiety in both human (Baas, Nugent, Lissek, Pine, & Grillon, 2004; Greenwald, Bradley, Cuthbert, & Lang, 1998; Lang, 1995) and animal (Falls & Davis, 1995; Hitchcock & Davis, 1991; Kim & Davis, 1993) subjects, after the presentation of aversive stimuli. One desirable advantage of the acoustic startle paradigm as an experimental tool, is that the primary measure, the acoustic startle response, is a reflexive action rather than an operant response. The benefits of measuring a reflex rather than an operant response are two-fold, firstly because startle response will have a pre-existing baseline greater than zero, it can be either enhanced or diminished under experimental conditions, whereas any learned behaviour will have an initial baseline of approximately zero (Koch, 1999). Secondly, the acoustic startle response employs relatively straightforward and established circuitry in the lower brainstem, compared to

the more complicated cognitive processing required for operant responding (Koch, 1999).

Fear potentiated startle, as a phenomenon can be viewed as an anticipatory defensive behaviour (Davis, Falls, Campeau, & Kim, 1993; Misslin, 2003) . In the fear - potentiated startle paradigm, the conditioned stimulus produces a defensive behaviour in response to a potential threat. The antithesis of this behaviour would be defensive behaviour that is elicited by an actual and immediate threat. Fear - potentiated startle amplitudes peak, at a time consistent with the inter-stimulus-interval of the prior CS-UCS pairing (Davis et al., 1993). Thus illustrating the anticipatory nature of fear potentiated startle.

1. 3. Extinction of Fear

The continued presentation of a CS without the UCS will result in a gradual weakening of association culminating in the complete inability of the CS to produce the conditioned response in the subject (Pavlov, 1924). Prima facie, extinction of pavlovian conditioned fear is a form of suppression or internal inhibition (Pavlov, 1924) of a previously learned association between the CS and the UCS. Interestingly, it appears that the inhibition of the conditioned association is not permanent and the association may spontaneously recur (Pavlov, 1924). The proposition that extinction is merely the temporary inhibition of an excitatory conditioned response is given credence by the phenomenon of spontaneous recovery. Implicit in the concept of spontaneous recovery is the assumption that conditioned inhibition is stable over time and resistant to external disruptors. Rescorla (1969) however argues that the assumption that conditioned inhibition is more robust than conditioned excitation, is not supported by empirical evidence.

Conversely, it has been postulated that rather than the mere suppression of a conditioned association, extinction is actually another learned association, this time between the CS and the absence of the UCS. Konorski (1948) proposed that the excitatory neural connection governing the conditioned reflex gradually diminishes over time as the neural connection governing the inhibitory connection strengthens. In this context the conditioned association (CS→ UCS) is retrieved with each presentation of the CS in the original training context. Extinction learning is simply the pairing of the CS with the Non-UCS, in the original training context (Bouton, 1993). As extinction training unfolds the number of exposures to the CS→ Non-UCS pairing increases and the retrieval of that association begins to interfere with the retrieval of the initial CS→ UCS association. Subsequent phenomena, such as spontaneous recovery and renewal, can be interpreted as a failure to retrieve the extinction association (Bouton, 1993).

1. 4. Neural Circuitry of Fear

Pavlovian conditioning develops a cue-specific fear in the subject, as opposed to a non-specific or generalised fear state (Rosen & Schulkin, 1998). This suggests amygdala involvement in the neural basis of pavlovian conditioned fear, as amygdala lesions have been found to block the acquisition of cue-specific fear potentiated startle (Hitchcock & Davis, 1991). The amygdala is the brain structure responsible for receiving the sensory inputs that arise from a fear-evoking stimulus and for producing the requisite behavioural response (Rosen & Schulkin, 1998). In a pavlovian conditioned paradigm such as fear-potentiated startle, stated in a rudimentary fashion, the external input from the

CS is filtered by the amygdala and the appropriate conditioned response is activated (LeDoux, 1998). Specifically, with the acoustic startle response, the auditory input is transmitted via the cochlear nuclei complex to the caudal pontine reticular nucleus (PnC) and subsequently to the motor neurons which initiate the startle response (as summarised in (Koch, 1999)). Synaptic transmission between the PnC and the amygdala appears to be the point at which conditioned fear enhances the motoric startle response (Davis et al., 1993). The basolateral nucleus of the amygdala receives sensory input from all sources; in turn the basolateral nucleus projects this information to the central nucleus of the amygdala. The central amygdaloid nucleus projects to the PnC of the acoustic startle circuit. Learned fear associations about the CS, are triggered by sensory input and this is subsequently relayed through the nuclei of the amygdala to the PnC of the startle circuit (Davis et al., 1993). In this way conditioned fear produces the enhancement of the acoustic startle response characteristic of fear potentiated startle.

The importance of the amygdala in fear neural circuitry extends far beyond its' seemingly simple relay-role in fear-potentiated startle. As previously alluded to, the amygdala is necessary in the acquisition of cue specific fear- potentiated startle and is also necessary for expression of the response (Hitchcock & Davis, 1991). Just as the amygdala appears important in both the acquisition and expression of fear potentiated startle, so too does dopamine within the amygdala. Kokkinidis and Waddington-Lamont (1998) found that the infusion of the dopamine D1 receptor antagonist SCH23390 into the amygdala complex resulted in decreased expression of fear-potentiated startle. Similarly, Guracci

and colleagues (1999) found that infusions of SCH23390 into the amygdaloid complex decreased acquisition and expression of freezing, whereas SKF82958 (a D1 receptor agonist) increased acquisition and expression of freezing. Furthermore, amphetamine, a dopamine agonist, has been reported to potentiate the response of the amygdala during the perceptual processing of fearful faces, in human subjects (Hariri et al., 2002).The amygdala receives the majority of its' dopaminergic projections from a region known as the ventral tegmental area (VTA), located on the floor of the mesencephalon. 6–OHDA lesions of the VTA were found to reduce dopamine levels in the amygdala by around 90% (Oades & Halliday, 1987). As dopamine appears to play an important role in the functioning of the amygdala in conditioned fear, logic would suggest that the major contributor of amygdaloid dopamine, namely the VTA, should be investigated in relation to conditioned fear.

1. 5. Ventral Tegmental Area

The amygdala although important in conditioned fear, is however, just one component of an interconnected network involved in the acquisition, expression and extinction of conditioned fear. The ventral tegmental area (VTA) is another such constituent of the neurocircuitry involved in conditioned fear. Connectively, the amygdala and the VTA interact via the mesolimbic pathway. The VTA projects dopamine fibres directly to the amygdala and indirectly receives dopamine projections from the amygdala via the pre-frontal cortex (Oades & Halliday, 1987).

The ventral tegmental area, is a mid - brain region that is known to play a crucial role in the impetus behind behaviour. Located on the floor of the

mesencephalon the ventral tegmental area is medial to the substantia nigra and ventral to the red nucleus (Swanson, 1982). The estimated 14,000 cells of the VTA mainly consist of small to medium sized neurons. Approximately two-thirds of these cells are dopaminergic, making the VTA one of the most dopamine rich regions of the midbrain. The efferent projections of the VTA can be divided into three major components. The first of these pathways projects through the medial forebrain bundle to a variety of forebrain regions including, among others, the nucleus accumbens and the amygdala. The second major projection is to the thalamus while, the third projection descends to the periaqueductal grey area, the locus coeruleus and the median raphe nucleus (Swanson, 1982).

The VTA with its' abundant source of mid-brain dopaminergic neurons, has been implicated in behavioural sensitisation, drug dependence and self stimulation studies (for a review see (Bonci, Bernardi, Grillner, & Mercuri, 2003)). A massive 70 % of all VTA projections are dopaminergic (Swanson, 1982) highlighting the importance of the VTA in the distribution of dopamine.

1. 6. Dopamine, Fear-Potentiated Startle & the Mesolimbic System

The mesolimbic pathway is a dopaminergic projection originating in the VTA and projecting to the nucleus accumbens and the amygdala (Swanson, 1982, Oades & Halliday, 1987). The VTA is necessary for the expression of fear-potentiated startle (Borowski & Kokkinidis, 1996). NMDA-induced lesions of the VTA resulted in the inability of subjects to express an augmentation of fear in the presence of a previously conditioned stimulus (Borowski & Kokkinidis, 1996). In particular the dopaminergic projections of the VTA are crucial to the expression of conditioned fear as infusions of the dopamine $D_{2/3}$ receptor

agonist, quinpirole, into the VTA suppressed fear-potentiated startle (Borowski & Kokkinidis, 1996).

Connectively, the dopaminergic projections of VTA and their terminal regions are also heavily involved in the acquisition and expression of fear- potentiated startle. Quinpirole, a dopamine autoreceptor agonist which reduces the rewarding efficacy of VTA stimulation (Ranaldi & Beninger, 1994), has been found to block fear-potentiated-startle, when administered to the VTA (Borowski & Kokkinidis, 1996; Gifkins, Greba, & Kokkinidis, 2002). Hitchcock and Davis (1991) found that lesions in numerous places along the pathway from the central nucleus of the amygdala to the brainstem startle circuit blocked fear-potentiated startle. The mesoamygdaloid dopaminergic pathway is also critical for the retrieval of fearful associations created during fear conditioning (Nader & LeDoux, 1999). Inhibition of dopaminergic activity on this mesolimbic pathway interferes with the retrieval of the previously learnt CS → UCS association. The pharmacological inhibition of dopamine activity in the VTA or the basolateral amygdala (BLA) with either quinpirole or SCH23390 results in this retrieval impairment (Nader & LeDoux, 1999). Therefore, irrespective of the terminal or somatic nature of the dopamine neurons of the mesolimbic pathway, their inhibition results in the same impairment.

Electrical stimulation at a variety of levels in the VTA produced significant increases in acoustic startle amplitudes (Borowski & Kokkinidis, 1996). Stimulation of the VTA appears to produce the same hyperdopaminergic state in the rat brain that chronic administration of psychomotor stimulants does

(Watanabe, Morimoto, Nakamura& Suwaki, 1998). Dopamine receptor antagonists have been found to successfully moderate the behavioural effects of VTA stimulation (Watanabe, et al, 1998). Interestingly, the chronic systemic administration of dopamine agonist, methamphetamine, reduced the threshold of electrical stimulation required in the VTA to achieve pre-determined abnormal behaviours (Watanabe, et al, 1998).These findings emphasise the importance of hyperdopaminergic activity in the mesolimbic system and in particular in the VTA during the abnormal behaviour seen in both idiopathic and drug induced psychosis.

2 Psychomotor Stimulants, Fear & the Mesolimbic System

2. 1. Psychomotor Stimulants and Fear

Psychomotor stimulants juxtapose reward and aversion. Commonly abused for their euphoric properties, psychomotor stimulants such as cocaine and amphetamine are capable of inducing both joy and fear. These disparate effects are anatomically distinct yet causally similar. Intracranial microinjections of amphetamine into a variety of brain regions, showed an anatomical dissociation of the rewarding and aversive effects of the drug. The nucleus accumbens proved to be vital for the rewarding effects, while the area postrema demonstrated its' importance in the aversive effects of amphetamine (Carr & White, 1986).

The capacity of psychomotor stimulants to pharmacologically induce negative emotional states has proven to be a valuable research tool. Discovered

clinically (Young & Scoville, 1938), the prolonged administration of amphetamine results in the subject developing a state similar to the paranoid psychosis observed in those with schizophrenia (Angrist, Sathananthan, Wilk, & Gershon, 1974; Griffith, Cavanaugh, Held, & Oates, 1970). Characteristic of this type of psychosis are paranoid ideations, themselves indicative of exaggerated and abnormal fear in the individual. Thus chronic amphetamine exposure has been utilised in animal research as a paradigm of abnormal fear. Additionally, the psychosis induced by prolonged amphetamine abuse can be reinstated spontaneously, by re-exposure to amphetamine or by exposure to psycho-social stressors (Griffith et al., 1970; Sato, Numachi, & Hamamura, 1992; Yui, Goto, Ikemoto, & Ishiguro, 2000).The latent continuity of amphetamine psychosis is perhaps indicative of an underlying and permanent inability within the individual to extinguish inappropriate associations.

2. 2. Systemic Studies

Systemic administration of psychomotor stimulants appears to globally enhance fear. Prolonged administration of cocaine increased the expression of conditioned fear in subjects evaluated with a FPS paradigm (Willick & Kokkinidis, 1995). Acute administrations of cocaine, d-amphetamine and SKF38393 (a D1 receptor agonist) prior to extinction training retarded the subjects' ability to cease fearing the CS (Borowski & Kokkinidis, 1998). The curbing of extinction learning by psychomotor stimulants suggests the drugs possess fear - enhancing properties. Chronic administration of cocaine prior to extinction training was also found to produce deficits in extinction learning (Willick & Kokkinidis, 1995). Furthermore, the re-administration of a single dose of either cocaine or SKF38393 is sufficient to reinstate previously extinguished

conditioned fear (Borowski & Kokkinidis, 1998). Pre- exposure to cocaine produces long-lasting changes to fear potentiated startle (Gordon & Rosen, 1999). During the withdrawal period, following chronic administration of cocaine, subjects showed an increase in startle responses both to a noise burst and to a conditioned stimulus. The increase in startle is indicative of heightened anxiety during the withdrawal period. It also suggests that the stimulant is in some way facilitating the fear association, to an explicit cue, over a prolonged period of time.

2. 3. Pharmacological Actions of Amphetamine

The primary action of amphetamine in the central nervous system is to potentiate the release of dopamine. The acute effects of amphetamine on dopaminergic neuronal function are dual in nature. Pre-synaptically, the stimulant causes an increase in the release of dopamine from the neuron, while concurrently decreasing dopamine reuptake by the pre-synaptic neuron (Lieberman, Kinon & Loebel, 1990). Consequently, the acute actions of amphetamine are an increase of dopamine levels in the synaptic cleft. The psychomotor stimulant has been found to affect the dopaminergic neurons of the CNS in both an excitatory and inhibitory manner (Shi, Pun, Zhang, Jones & Bunney, 2000). This inhibition and excitation is mediated by the dopamine D_2 receptor. The excitatory effect of amphetamine on the dopamine cell is evident when the D_2 receptor is blocked and the inhibitory effect is seen when it modulates dopamine cell firing (Shi et al., 2000). This finding suggests that amphetamine has a dual effect on dopamine cells, namely DA mediated inhibition and non-DA mediated excitation.

3 Psychomotor Sensitisation

3.1. Sensitisation

Sensitisation occurs when chronic exposure to a stimulus, produces long-lasting enhancement of its normal physiological effects. The phenomenon of pharmacological sensitisation can be elicited following repeated administration of a particular drug by a single challenge presentation of that substance (for a review see Vanderschuren & Kalivas, 2000). Pre-exposure to that particular drug, will ensure that the individual is sensitised to its' effects and consequently the challenge-exposure will result in heightened effectiveness of the substance. The sensitisation process implies that the threshold for activation has become lower over time (Rosen & Schulkin, 1998). The phenomenon of sensitisation is indicative of hyperexcitability of the neurocircuitry involved.

Sensitisation is a long – lived phenomenon. The enduring nature of neurochemical sensitisation is supported by evidence that individuals, who have previously attained a state of methamphetamine induced psychosis, will relapse given a single dose of methamphetamine following a period of abstinence (Sato, Chen, Akiyama & Otsuki, 1983). The acute dose of methamphetamine given may be markedly smaller than doses taken during the period of abuse, none of which were sufficient to induce psychosis. The relapse into the psychotic state is indicative of long-lasting sensitisation to the effects of the

stimulant. Interestingly, the concurrent administration of the neuroleptic haloperidol, a potent dopamine receptor blocker, prevented relapse when the subject was given a challenge-dose of methamphetamine following a period of abstinence (Sato, et al., 1983). Following the discontinuation of haloperidol, a single dose of methamphetamine was sufficient to re-induce psychotic symptoms. The longevity of behavioural sensitisation is such that even several years later, a single presentation of the abused drug can induce psychosis (Sato, et al., 1983). The long-lived nature of sensitisation indicates that psychomotor stimulants modify brain functioning for a prolonged period of time following their administration.

The dormant nature of behavioural sensitisation bears strong similarity to the unseen development of schizophrenia and like many psychotic episodes can be triggered either by an external stressor or pharmacologically. Re-exposure to a chronically administered drug is not the only catalyst of behavioural sensitisation. Other factors such as stress may trigger behavioural sensitisation (Sato, Chen, Akiyama & Otsuki, 1983, Yui, Goto, Ikemoto & Ishiguru, 2000). Interestingly, stress may exacerbate sensitisation to psychomotor stimulants. Castner and Goldman-Rakic (1996) reported that the more baseline “stress” an animal exhibited, the more pronounced the effect of amphetamine on the abolition of stereotypic behaviours during sensitisation. In addition to this, they also reported an exacerbation of stressful responses by the subjects during amphetamine sensitisation. These stressful responses included behaviours such as self - biting and saluting.

3. 2. Neuroanatomical Substrates of Sensitisation

The VTA has proven to be necessary for both the development and inhibition of sensitisation to psychomotor stimulants (Jones, Kornblum & Kauer, 2000). Acute infusions of amphetamine into the VTA produce no discernable increase in locomotor activity (Kalivas & Weber, 1988) in contrast to the effects of peripheral administration, where an increase in locomotor activity is legend. Interestingly, a single infusion of amphetamine to the nucleus accumbens produces a significant and dose-dependent increase in locomotor activity (Carr & White, 1986, Cador, Bjijou & Stinus, 1995). The total dissociation of these two regions and their role in behavioural sensitisation is further exemplified by the chronic administration of psychomotor stimulants. Repeated intra-cranial amphetamine infusions in the ventral tegmental region will result in long-lasting changes to the sensitivity of that region. A challenge presentation of amphetamine, either peripherally or intra – cranially, following chronic administration results in heightened behavioural activity (Kalivas & Weber, 1988, Hooks & Jones, 1992, Cador & Bjijou, 1995). Both cocaine and amphetamine chronically administered intra-cranially to either the A9 (substantia nigra) region, or the A10 (ventral tegmental area) resulted in a potentiation of the psychomotor stimulant effects when administered peripherally. However, intra - cranial administration of the stimulants to the terminal regions of the dopamine cells of the A9 and A10 regions, namely the nucleus accumbens, the striatum and the lateral ventricle, did not result in any augmentation of the peripheral drug administration(Kalivas & Weber, 1988).

4 Rationale and Hypothesis

A dual theme gains clarity from within the literature surrounding the neural basis of abnormal fear. The potentiation of dopamine, whether organically or synthetically, and activity of the mesolimbic system both seem to play a crucial role in abnormal fear. More specifically, two areas within the mesolimbic system, namely the ventral tegmental area (VTA) and the basolateral amygdala (BLA), emerge as a key regions underlying the behavioural manifestations of abnormal fear. An increasing amount of evidence begins to converge on the hypothesis that the VTA and its dopaminergic neurons are critical to the acquisition of conditioned fear, whilst the BLA is important in the processing, responding and extinction of fearful stimuli. However, very little is known about how the dopaminergic neurons of the mesolimbic system are influencing the extinction of fearful associations . Therefore, by using a dopamine agonist whose pharmacological actions are known, namely amphetamine, we can hope to uncover more about the actions of dopamine in the VTA and the BLA and their ramifications on the extinction of conditioned fear. Amphetamine has previously been found in systemic studies to (Kumar, 1971) retard fear extinction. In particular the aim of this study is to investigate the effects of amphetamine, infused into the VTA and the BLA , on the extinction of pavlovian conditioned fear responses. It is hypothesised that amphetamine administered to the VTA, the base of the mesolimbic system, immediately prior to extinction will significantly impair the extinction of a previously conditioned fear association. Whereas the effects of a central infusion of amphetamine to the

BLA are likely to differ from those of the VTA due to the contrasting terminal nature of the dopamine neurons in this region. In addition, the study will also seek to investigate a possible link between the behaviourally sensitising effects of chronic amphetamine administered to the VTA and the BLA and extinction.

5 METHOD

5. 1. Subjects

Male Wistar rats (University of Canterbury) weighing between 300 - 400g at the time of surgery, served as subjects in this study. The animals were group - housed throughout the study on a 12 hour light/dark cycle, with the light cycle beginning at 0800 and the dark cycle beginning at 2000. Both the light and dark cycles took place within a constant climatically controlled (21°C) colony environment. Behavioural testing occurred during the light portion of the cycle. Throughout the study subjects had free access to food and water.

5. 2. Apparatus

An acoustic startle system purchased from MED Associates (Fairfield, VT) was used for the fear conditioning, extinction, and potentiated acoustic startle testing. The acoustic startle system was comprised of four chambers and associated stimulus generators for light, shock and noise. All stimulus generators were controlled by a computer with purpose design software. Each melamine chamber (internal dimensions: 60 x 56 x 34 cm) was lined with sound attenuating acoustical foam which was 2.5 cm thick. During testing the subjects were enclosed in an animal holder (interior dimensions of cage: 16.5 x 8 x 9 cm) to restrict their movement. All four walls of the animal holder were made of horizontally placed steel bars (4.5 mm in diameter on floor, 2.5 mm in diameter on walls and roof, all spaced 15 mm apart). To reduce the likelihood of the animal damaging their head - cap during testing, wire mesh was placed over the bars on the roof and on the sides of the cage. The animal holding cage within

the chamber was mounted on MED associates' accelerometer -based transducer platform (25 x 11.5 x 4.5 cm). The movement of the subject was translated into proportional variations in voltage output and was filtered and amplified before being measured by a MED Associates analogue-to-digital converter (ADC) card, a component of the computer that controls stimulus presentation. The house-light (#1820; 28 volt, 2.8 watt) and horn tweeter (6 cm) in each of the chambers was situated in the centre of the back wall. The startle stimulus was a 100 ms white noise burst, with a rise-decay time of 10 ms. The startle stimulus was produced by a MED Associates ANL 925 Programmable Audio Stimulator, amplified by an ANL 925A Audio Amplifier and presented through the horn tweeter in each chamber. Ambient noise in the chambers is 55 dB as measured by a Simpson (model 860; Elgin, IL) sound level meter (A-scale). All stimuli presentations were controlled by MED Associates software.

Ambulatory activity was tested in a plexi - glass chamber (350mm width x 380mm height x 337mm depth). The chamber was raised 20 mm above the ground and the base of the chamber is a stainless steel plate (330mm x 320mm). Movement was measured via two sensors diagonally attached to the sides of the chamber. The sensors were positioned 200mm from the floor of the chamber. The movement measured by the sensors was controlled by an activity monitor (CFP 8181, Wiltons) and recorded on an electronic counter (Model 54417, Lafayette Instrument Co., Indiana).

5. 3. Procedure

5. 3. 1. Surgery

Pre - operatively, the subjects were given an intra - peritoneal injection of atropine (12mg/kg), this was prevent the accumulation of salivary and bronchial secretions during surgery. Thirty minutes later, rats were anaesthetised with a bodyweight injection sodium pentobarbitone (90mg/kg). They then received a subcutaneous injection of ketofen (10mg/ml) to the neck region, this served as an analgesic with anti-inflammatory properties. Prior to surgery the rats also received a 0.2ml injection of mepivacaine (20mg/ml) to the suture site, this acted as a local anaesthetic. The rats were then placed on a stereotaxic instrument (Stoelting, Wood Dale, IL) and bilaterally implanted with 22 – gauge stainless steel guide cannulae (C313G, Plastics One, Roanoake, VA). The cannulae were implanted on a 10° angle above the VTA, with the measurements from bregma, AP - 4.8, ML $\pm$ 2.5, DV -7.5, and with measurements for the BLA from bregma, AP – 2.8 , ML $+ 4.8$, DV – 8.2. The guide cannulae were be secured in place by four stainless steel jewellers' screws (Lomat, Quebec, Canada) and dental cement. A recovery period of seven days elapsed post surgery and prior to the commencement of testing.

5. 3. 2. Acoustic - Startle Screening

Post surgery and prior to the fear - conditioning rats were tested for their individual acoustic - startle reflex. The acoustic - startle reflex was measured over two days, in test sessions consisting of three blocks of 10 noise presentations with a variable inter-stimulus-interval (ISI) of approximately 20s. On each day, animals were placed in the test chambers where they received a

5 minute acclimation period followed by a test session of 20 minutes. In the initial screening session all rats were exposed to white noise bursts of 95dB (100ms). Depending on the results of the first session the decibel level was then adjusted for each individual, to achieve the desired level of startle response. Although, the individual decibel level varied, the range was generally be between 90 and 100dB.

5. 3. 3. Fear Conditioning

Following the selection of an appropriate decibel level, subjects were then given a session of fear - conditioning in the startle apparatus. Fear - conditioning was comprised of three sections; each section in turn consisted of 10 pairings of the light (CS) and 600µA foot - shock (UCS). Thus, a total of 30 pairings of the CS and the UCS were used to produce a Pavlovian - conditioned fear of the light stimulus. In this test design the ISI was fixed at 56s and the length of the stimulus presentations were 3500ms for the light presentation and 250ms for the shock presentation.

5. 3 .4. Group design

Part a : Extinction & Infusion

Group	N	Implanted	Drug	Extinction
Control II	7	VTA / BLA	Saline (x3)	Extinction
Experimental I	10	VTA	Amph. (x3)	Extinction
Experimental II	8	VTA	Amph. (x3)	No Extinction
Experimental III	7	BLA	Amph (x3)	Extinction

Part b: Psychomotor sensitisation

Group	N	Implanted	Pre. Infused	I.P. injection	Reason
Control I	7	_____	_____	Saline	Effect of injection stress and normal ambulatory activity
Control II	7	VTA / BLA	Saline	Amph.	Effect of amph. on ambulatory activity
Control III	10	VTA	Saline	Amph	No sensitisation
Exptal I	10	VTA	Amph.	Amph.	Sensitisation
Exptal II	8	VTA	Amph.	Amph.	Sensitisation
Exptal III	7	BLA	Amph.	Amph.	Sensitisation – terminal vs. cell body

5. 3. 5. Fear - Potentiated Startle Test

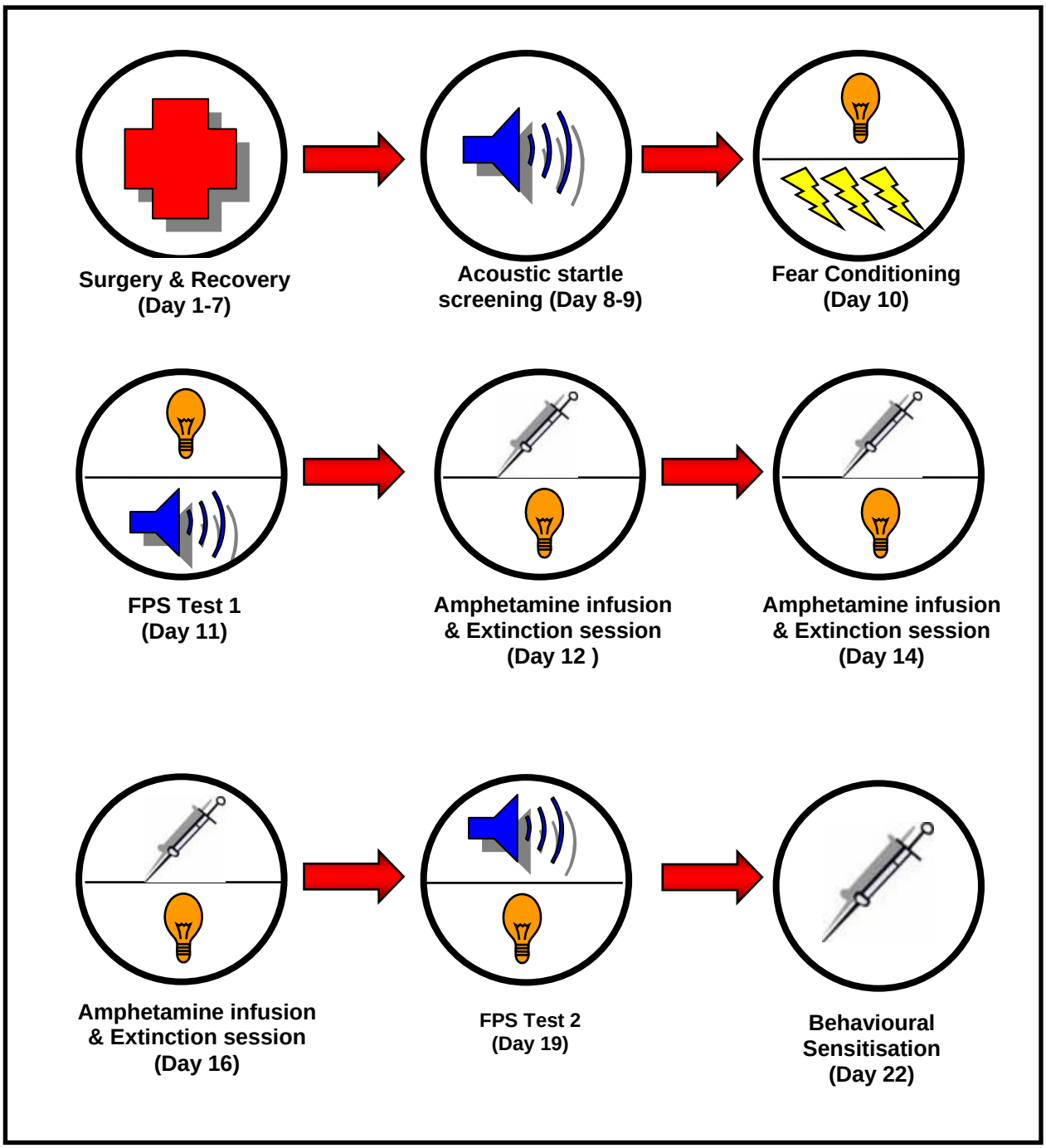
The subjects were placed in the startle chamber for a five minute acclimation period prior to the onset of the startle test. The test itself consisted of three different trial blocks. The first block was the presentation of 20 white noise bursts (100ms) with an inter-stimulus-interval (ISI) of 26 seconds. This block was used to establish the baseline acoustic startle reflex of the subject. The second block of trials were composed of 5 white noise presentations with a 26s ISI. This second series of noise alone presentations was used as a baseline comparison for the third and final series. The third block of trials is comprised of five pairings of a 100ms white noise burst and a 3500ms light (CS) presentation. The difference in acoustic startle reflex between the block of 5 trials with noise alone and the trials with the noise paired with the light, was used to calculate the augmentation of startle in the presence of the conditioned stimulus.

5. 3. 6. Infusion and Extinction

Following the first fear - potentiated startle test, rats received either an intra-VTA or an intra-BLA infusion of d-amphetamine or saline and extinction training. The dummy cannulae (C313DC, Plastics One) were removed and 28-gauge (0.36mm) stainless steel infusion needles (C3131, Plastics One) were inserted so that the tip of the needle protruded 1mm below the base of the guide cannulae. The infusion needles were attached via polyethylene tubing (PE20, Plastics One) to a 2 - µl Hamilton Syringe. The tube was preloaded with either physiological saline or d-amphetamine (2.5µg/0.5µl). The d-amphetamine (Sigma, Castle Hill, New South Wales, Australia) was dissolved in physiological saline mixture comprised of 2.5µg/0.5µl. Via the polyethylene tubing, the bilateral infusion of 0.5µl of either d-amphetamine or saline took 1 minute. A two-minute period after the infusion was allowed to elapse before the infusion needles were removed and the dummy cannulae were reinserted. The purpose of this two-minute period was to allow time for the drug to be absorbed into the designated area. Throughout the infusion the subjects were held lightly in a towel.

Immediately after the replacement of the dummy cannulae the subjects were placed in the either the startle chambers or placed back in their home cages. For those placed in the startle chambers, they then received extinction trials. Following a five – minute acclimation period those receiving extinction were then presented three blocks of ten light presentations. In each of these blocks the light will be presented for 3500ms with a fixed 56s ISI.

Fig. 5.1. Diagrammatic representation of procedure used.



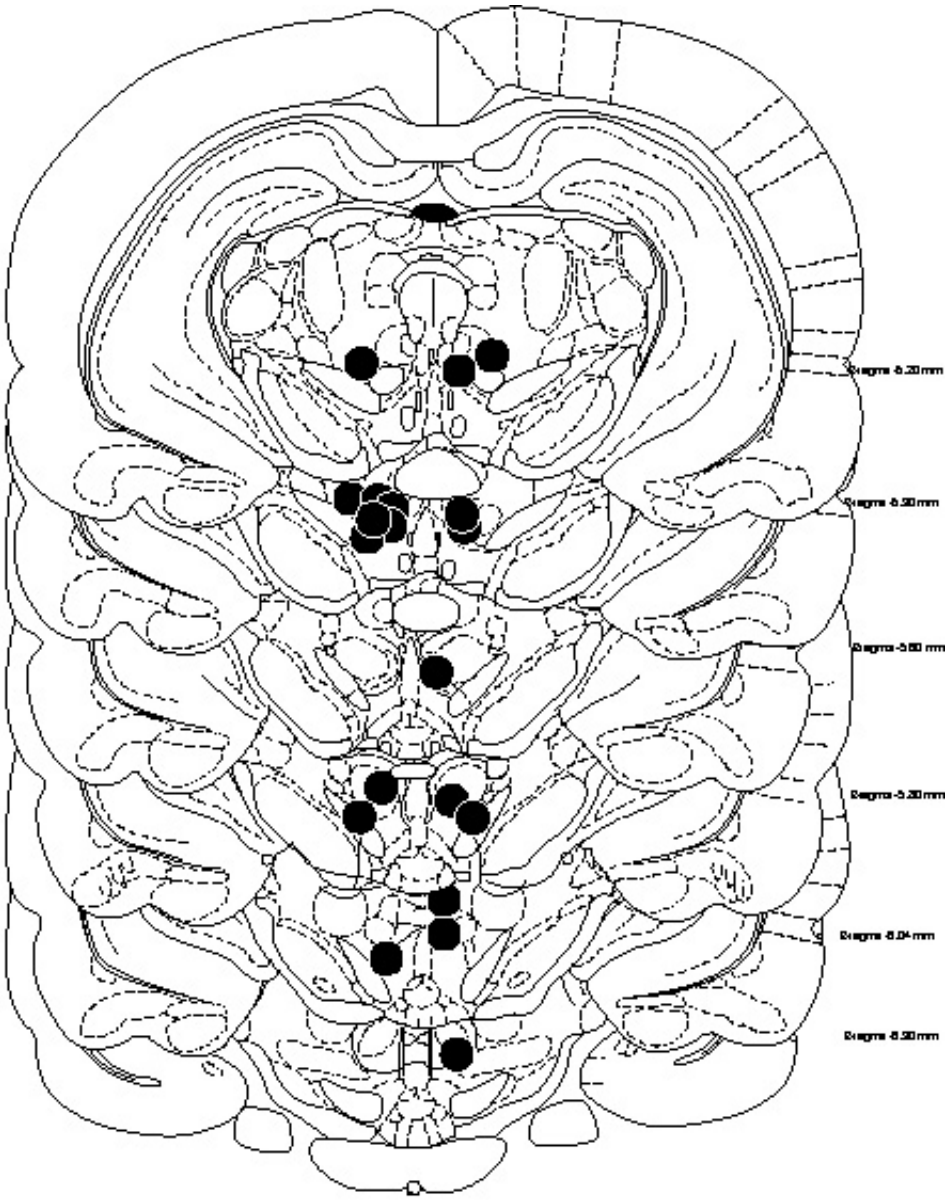
5. 3. 7. I. P. Injection and Behavioural Sensitisation

Animals were administered a 2 mg/kg dose of either d-amphetamine or physiological saline intra-peritoneally. The subjects were then placed in the locomotor chamber for an hour and their locomotor activity was be recorded in 10 minute intervals.

5. 4. Histology

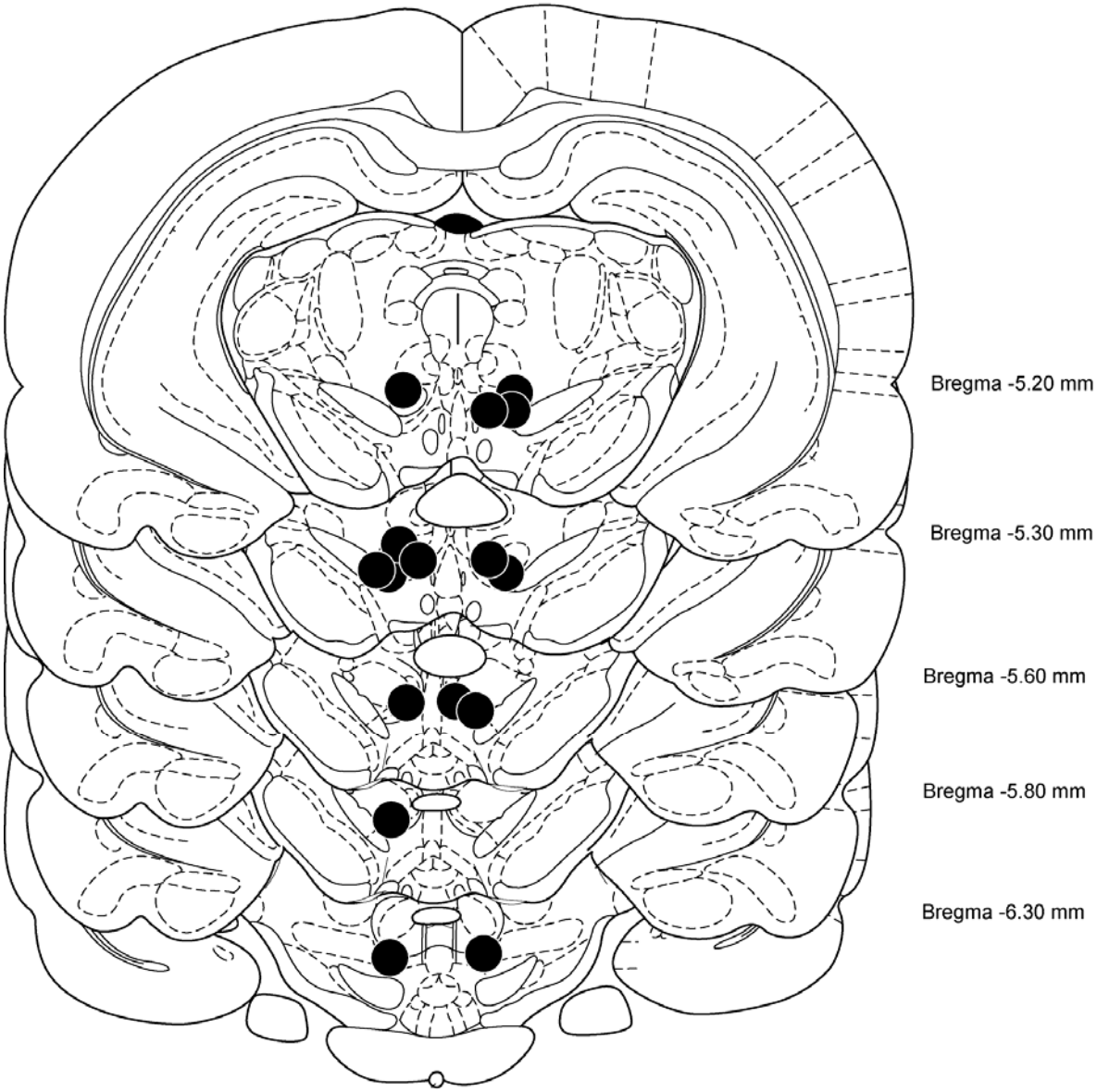
At the end of testing subjects were overdosed with sodium pentobarbitone and perfused intra-cardially with saline, followed by Formalin (10%). The brains were then removed and stored in a Formalin (10%) solution for two days before being transferred to a 70% sucrose solution for several weeks. The brain were then frozen (-24˚) and coronal sections (50 µm) of the area of interest were sliced. These coronal sections were mounted on gel coated slides and stained with cresyl violet. The placement of the guide cannulae were then confirmed microscopically. One subject (A7) from mixed control group II, two subjects (A5 and A12) from the BLA/Amphetamine + Extinction group, and 1 animal (#54) from the VTA/Amphetamine + No Extinction group lost headcaps and did not complete testing. One subject (#24) from experimental group one VTA/ Amphetamine + Extinction, had incorrectly placed cannulae and was not included in the analysis. Cannulae placements of subjects included in the data analysis are depicted in Figures 5.4.1.-5.4.3.

5. 4. 1. Placement verifications for Experimental Group I (Bilateral VTA implants)



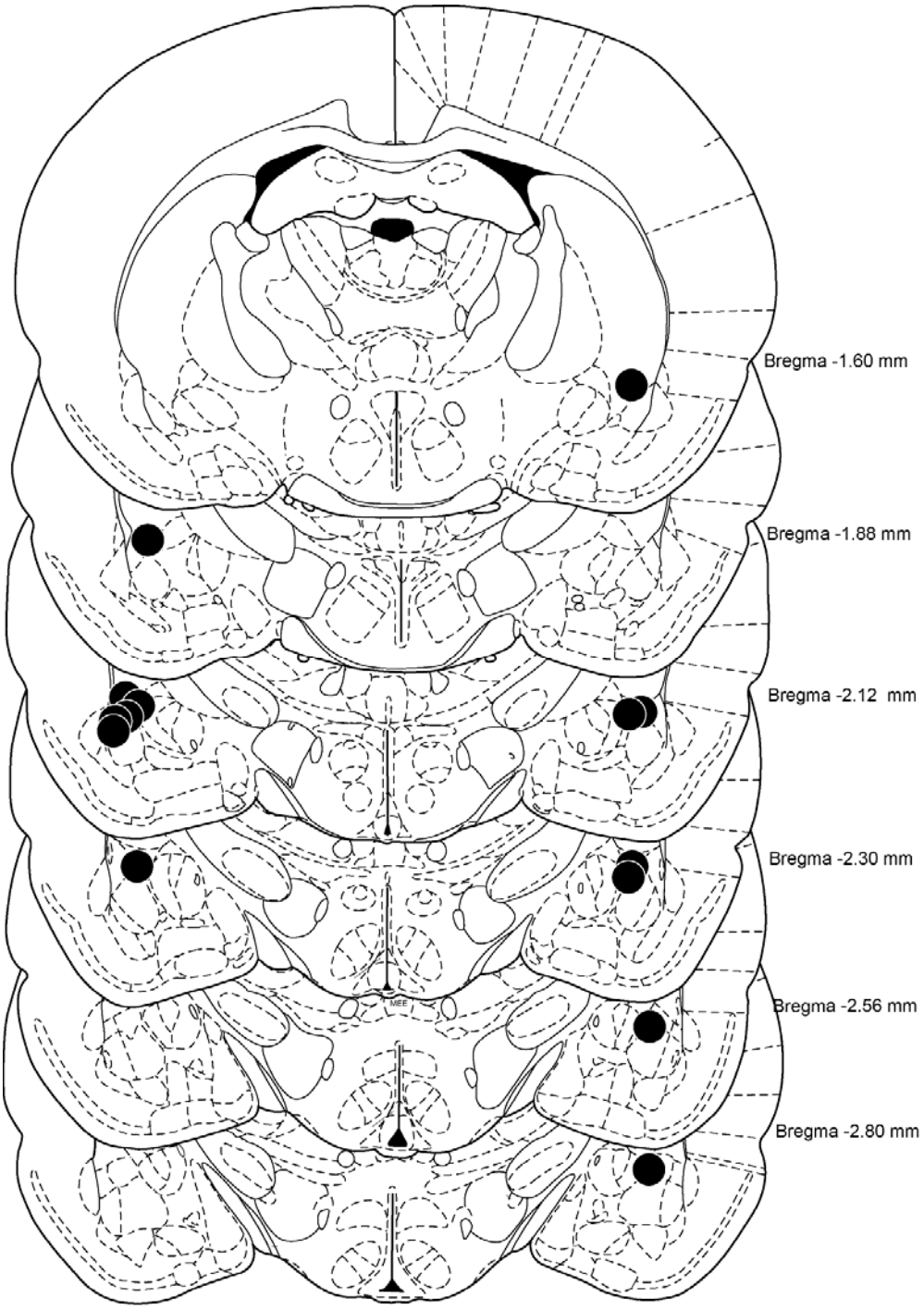
A schematic depiction of guide cannulae locations for Experimental Group 1 – Amphetamine / VTA + Extinction (N = 10) Bilateral VTA implants, guide cannulae were implanted at a 10 ° angle, with the measurements from bregma, AP - 4.8, ML ± 2.5, DV - 7.5, aimed 1mm above VTA. Representative sections (Paxinos & Watson, 1998)

5.4.2. Placement verifications for Experimental Group II (Bilateral VTA implants)



A schematic depiction of guide cannulae locations for Experimental Group 2 – Amphetamine / VTA + No Extinction (N = 8)Bilateral VTA implants, guide cannulae were implanted at a 10 ° angle, with the measurements from bregma, AP - 4.8, ML ± 2.5, DV - 7.5, aimed 1mm above VTA. Representative sections (Paxinos & Watson, 1998)

5. 4. 3. Placement Verifications for Experimental Group III (Bilateral BLA implants)



A schematic depiction of guide cannulae locations for Experimental Group 3 – Amphetamine / BLA + Extinction (N = 7) Bilateral BLA implants, guide cannulae were implanted at a 10 ° angle, with the measurements from bregma, AP - 2.8, ML ± 4.8, DV - 8.2, aimed 1mm above BLA. Representative sections (Paxinos & Watson, 1998)

6 RESULTS – PART A

6. 1. Acoustic Startle and Selected Decibel Levels

The acoustic startle levels of individual animals were not significantly different across groups during baseline screening sessions, F (4, 36) = 0.19, p = 0.94. Thus, any differences seen in startle levels following experimental manipulations were not the result of variable baseline levels. Additionally, the decibel levels selected for each subject did not differ significantly between the experimental groups, F (4, 36) = 0.83, p = 0.51. The noise burst intensity to measure acoustic startle varied between 92 – 98 dB, with an average level of 94. 85 dB.

SECTION ONE: Effect of Intra-VTA Amphetamine on Extinction

Control Group 1: (*Chronic saline infusions prior to extinction*)

Subjects who received three intracranial infusions of saline served as a control group (Control Group 1, N = 7) for animals who received three intracranial infusions of amphetamine prior to extinction sessions. Within the group each subject served as its own control, providing an acoustic startle score for the FPS test ascertaining acquisition of conditioned fear, and following extinction sessions, for the FPS test ascertaining extinction of conditioned fear. A 2 TEST (acquisition / extinction) X 2 STIMULUS (Noise alone / Light + Noise) repeated measures showed a significant interaction between the two main factors, Test and Stimulus, F (1,6) = 7.78, p < 0.032, for the control group. There was no significant alteration in baseline acoustic startle responding throughout the

experimental procedure, subjects showed consistent acoustic startle levels in the noise-alone component of both the initial and final fear test, F (1, 6) = 1.34, p = 0.29 . The control group acquired significant fear of the light following pavlovian fear -conditioning trials, F (1, 6) = 10.78, p< 0.02, and displayed augmented acoustic startle responses in the presence of the light on the initial fear test. However, after receiving three extinction sessions of 30 light alone presentations, with saline infusions prior to each one, the control subjects did not show significant fear of the light on the final fear test, F (1,6) = 0.61, p = 0.47. These results demonstrate normal extinction learning, where the control subjects had effectively extinguished their previously acquired fear of the light, following the continued presentation of the light (CS) without the corresponding shock (UCS) during extinction sessions.

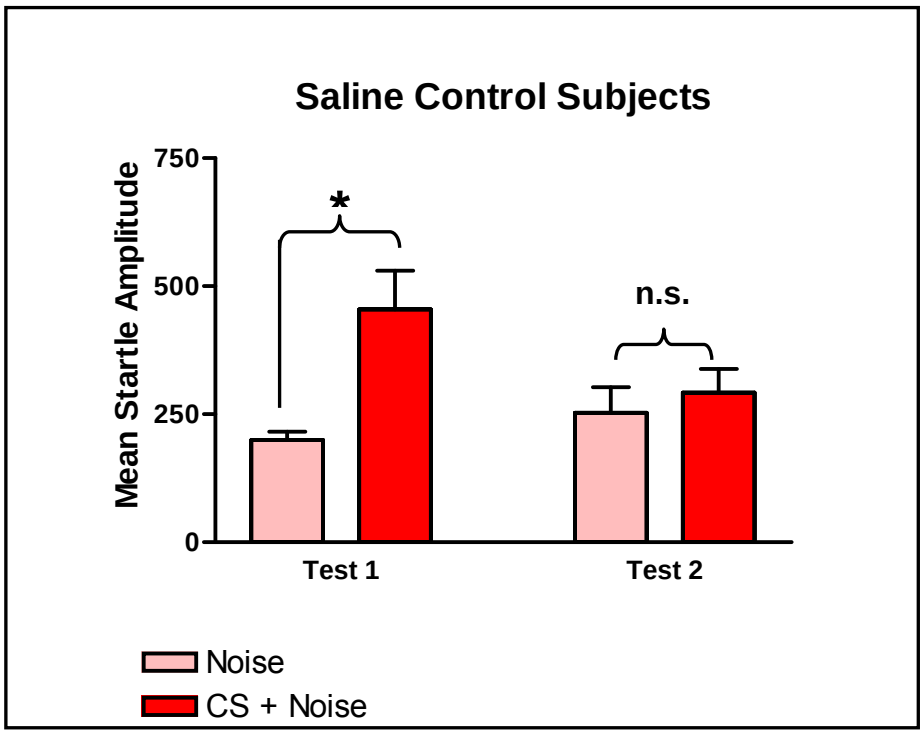


Fig.6.1. **Repeated- measures ANOVA results for subjects infused with saline (Control Group 1, N = 7). Significant difference seen in FPS Test 1 only, p < 0.05. FPS test 2, *n.s*.**

Experimental Group 1: (*Repeated intra-VTA amphetamine prior to extinction*)

Like control subjects, the subjects in Group 1 showed consistent acoustic startle levels in the noise-alone component of both the acquisition and extinction fear test, F (1, 9) = 0.0003, p =0.99, meaning baseline - startle responding did not change during the course of the experiment. In this design animals served as their own control subjects for within group comparisons. A repeated measures ANOVA yielded a significant Test X Stimulus interaction, F (1,9) = 7.22, p < 0.02. Experimental subjects acquired conditioned fear, as measured with fear-potentiated startle, on the initial fear test following conditioning trials F (1, 9) = 64.5, p < 0.00002. Following three extinctions sessions, prior to which each animal was infused bilaterally with 2.5µg d-amphetamine intra-VTA, the subjects were tested again for conditioned fear. The animals showed that they had retained their previously conditioned fear of the light, in spite of 90 unreinforced presentations of the light over three extinction sessions F (1, 9) = 12.5, p < 0.006. Unlike the control subjects, the experimental group did not show normal extinction learning indicating that repeated intra-VTA administration of d-amphetamine, prior to extinction sessions, impairs extinction of conditioned fear.

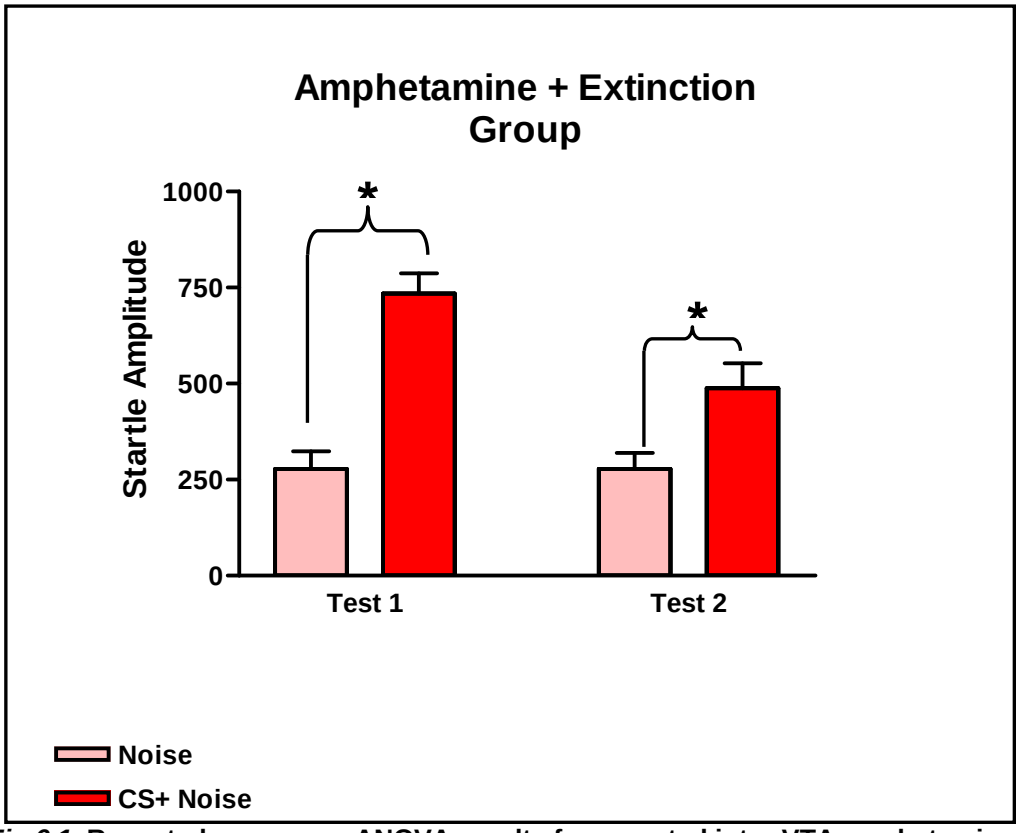


Fig.6.1. **Repeated- measures ANOVA results for repeated intra-VTA amphetamine subjects (Experimental Group 1, N = 10), FPS Test 1 & 2, both tests significant, $p < 0.01$**, $p < 0.0001$ ****.**

Experimental Group 1 vs. Control Subjects: (*Repeated intra-VTA amphetamine prior to extinction vs. Repeated saline infusions prior to extinction)*

As the experimental manipulation occurred after the first fear test, during the extinction sessions, any evidence of a difference between the two groups would only be apparent on the final fear test. It was hypothesised that there would be a significant difference between the group which received amphetamine infusions prior to extinction sessions and control subjects who received saline infusions. To assess the between group effects a 2 DRUG (saline/amphetamine) X 2 STIMULUS (Noise alone/ Light + Noise) ANOVA was conducted on the final fear test, showing a difference between the two groups

on the final FPS test that was approaching significance, F (1, 15) = 4.22, p < 0.058.

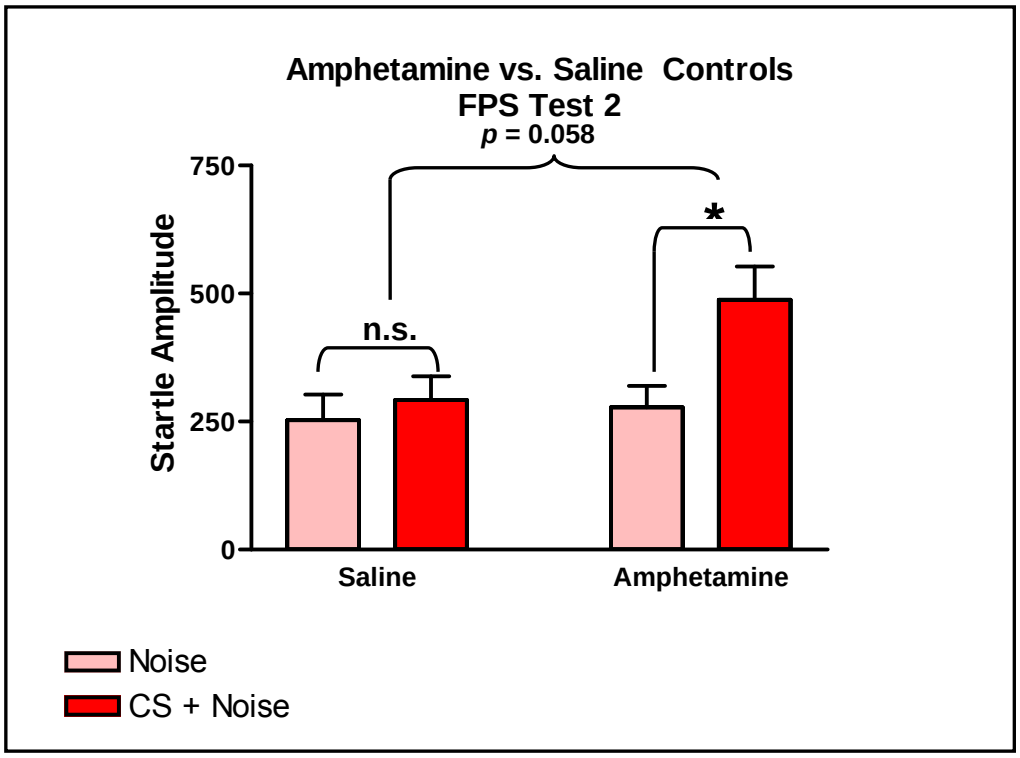


Fig.6.3. **Repeated- Measures ANOVA between Repeated AMP-VTA + Extinction (Experimental 1) and Repeated Saline + Extinction (Control 1)on FPS Test 2. Difference between the groups approaching significance.**

A simple effects analysis showed no significant difference between the two groups noise – alone component, F (1, 15) = 0.15, p = 0.71, meaning that baseline acoustic startle responding was comparable for each group. Conversely, there was a significant main effect between the two groups for the light + noise component, F (1, 15) = 5.05, p < 0.04, showing that the acoustic startle responses of the experimental subjects were significantly in excess of the startle levels exhibited by control subjects. Importantly, this difference was not the result of an initial variation in baseline between the two groups.

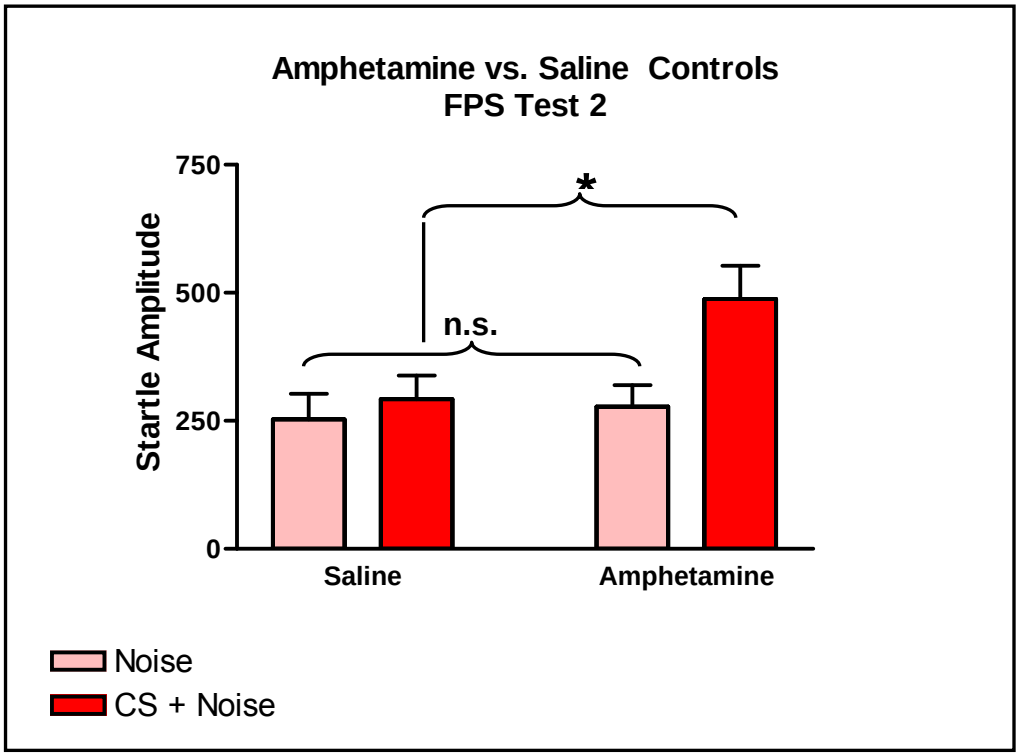


Fig.6.4. **Contrasted Stimulus effects. Noise alone and Light + Noise conditions on FPS Test 2, for AMP-VTA + Extinction (exp 1) and Sal + Extinction (con 1). One – way ANOVA for both groups on each Stimulus-Condition.**

The overall analysis indicated a difference between the two groups that was approaching significance on the second fear test, closer analysis showed that the two groups were responding significantly different in the presence of the conditioned stimulus (light). The experimental subjects were still showing significant fear of the light on the final fear test whereas the control subjects were not, with the controls showing normal extinction learning and the subjects who received amphetamine showing an extinction impairment.

S E C T I O N T W O : Effect of Repeated Intra-VTA Amphetamine on the Expression of Pavlovian Conditioned Fear.

This section examines whether the results seen in Section One of the study were the consequence of repeated d-amphetamine enhancing conditioned fear in subjects. To determine the effect of repeated intra-VTA amphetamine on the expression of Pavlovian conditioned fear, a group of animals (Experimental Group 2, *N* = 8) received fear conditioning and were tested for fear-potentiated startle. Following the confirmation from the FPS test, that the subjects had acquired fear of the conditioned stimulus, they were infused 3 times with d-amphetamine (2.5 µg bilaterally), with each infusion spaced 48 hours apart. The key difference between experimental group 2 and experimental group 1, is that after each infusion subjects in group 2 were returned to their home cages without extinction sessions. Consequently, the behaviour of the subjects in group 2 on the final fear test would indicate whether the repeated administration of amphetamine to the VTA was increasing the expression of pavlovian conditioned fear, thereby clarifying the extinction impairment seen in group 1.

Once again animals served as their own controls for within group measures. A 2 TEST (acquisition/expression) X 2 STIMULUS (Noise alone/ Light + Noise) repeated –measures ANOVA showed no significant Test X Stimulus interaction, F (1, 7) = 2.99, p = 0.12. Subjects showed acquisition of Pavlovian conditioned fear, with significantly augmented acoustic startle responses in the presence of the light on the initial FPS test, F (1, 7) = 28.7, $p < 0.001$. Subjects still showed significant fear of the conditioned stimulus on the final fear test, F (1,7) = 6.05, $p < 0.04$, after the three amphetamine infusions. Although, for both the first and second FPS tests subjects showed significant fear of the conditioned stimulus,

the magnitude of the augmentation of acoustic startle in the final fear test was somewhat smaller than the initial test. A similar outcome was also apparent in experimental group 1. The most likely explanation is that the 5 presentations of the unreinforced CS in the initial fear test slightly reduced the fear of the CS in subjects. However, the two groups still exhibited significantly enhance fear of the conditioned stimulus on the final fear test. Figure 2.1 below illustrates the stability of fear responding for Group Three across tests. The important point that is illustrated clearly in Figure 6.5., is that the amphetamine administration did not cause a significant increase in fear expression on the final fear test. Thus, the extinction impairment seen in group 1 cannot be attributed to repeated amphetamine enhancing fear expression in subjects.

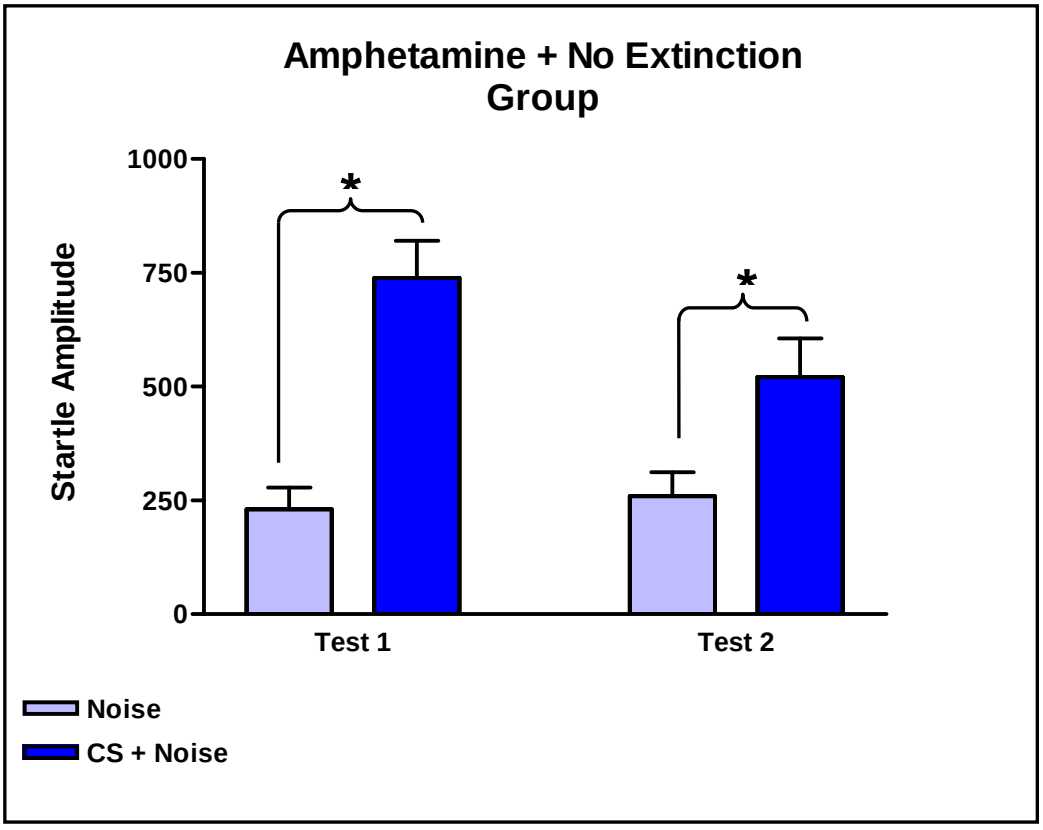


Fig.6.5 **Repeated- Measures ANOVA for within group effects for AMP-VTA + No extinction group (exp. 2) No significant difference between tests, both FPS tests show significant fear-induced elevations, *F* (1, 7) = 28.67, p < 0.0011**, *F* (1, 7) = 6.05, p < 0.04*.**

When compared to subjects who received extinction sessions, group 2 did not significantly differ from them in their acoustic startle responses on the final fear test. Baseline acoustic startle levels, in the noise-alone condition were similar across Groups 1 and 2, F (1, 16) = 0.24, p = 0.63. Interestingly, the augmentation of startle amplitudes elicited by the light for both FPS tests, for both groups did not differ from one another, F (1, 16) = 0.19, p = 0.66. A repeated – measures ANOVA yielded no significant difference between the two groups on either the initial fear test, F (1, 16) = 0.24, p = 0.63, or the final fear test, F (1, 16) = 0.19, p =0.66.

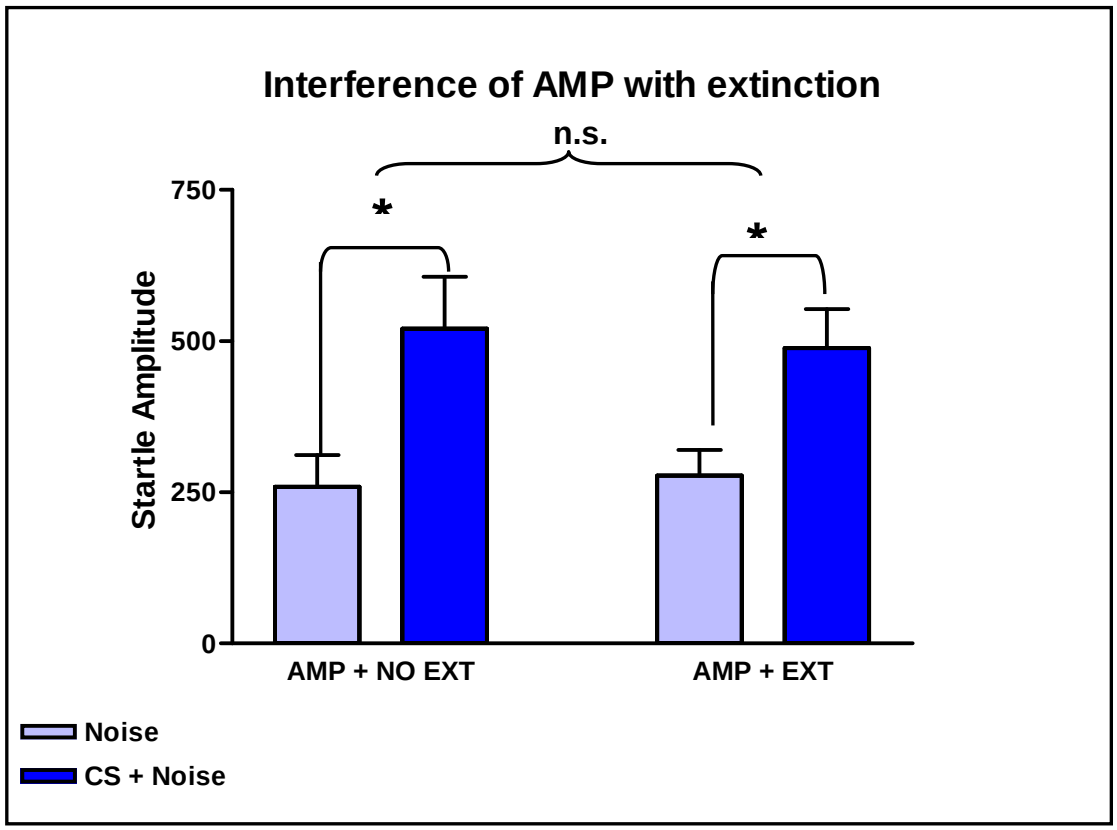


Fig.6.6. Repeated-Measures ANOVA. Comparison of FPS Test 2 both AMP-VTA groups, extinction vs. no extinction. No significant difference between groups. p = 0.66.

It appears that the responding of the two groups who received repeated amphetamine infusions, is similar irrespective of whether the subjects received extinction sessions. Furthermore as amphetamine is not enhancing the manner in which subjects express fear , it indicates that the extinction impairment is likely to originate from amphetamine's neural effects in the VTA on extinction learning

S E C T I O N T H R E E : Regional Dissociation of Repeated Intracranial d- amphetamine Administration on the Extinction of Conditioned Fear

The basolateral amygdala (BLA) plays a critical role in the acquisition, expression, and extinction of Pavlovian conditioned fear in rats (Kellett & Kokkinidis, 2004; Lindgren, Gallagher, & Holland, 2003; Nader & LeDoux, 1999). Pharmacological manipulation of the BLA NMDA receptors blocks both acquisition and extinction of conditioned fear (Lee & Kim, 1998; Maren, Aharonov, Stote, & Fanselow, 1996). Given the importance of the BLA in extinction learning we assessed the effect of repeated amphetamine infusion bilaterally into the BLA prior to extinction sessions. Again, subjects served as their own controls for within group measures, with a comparison of acoustic startle augmentation on the acquisition FPS test and the final fear test following 3 extinction sessions. No significant Test X Stimulus Interaction for Group Four, was yielded by a 2 X 2 repeated –measures ANOVA, F (1, 6) = 5.04, p = 0.07. Subjects who received repeated intra-BLA amphetamine showed significantly increased startle in the presence of the conditioned stimulus in the initial FPS test as expected, F (1,6) = 40.3, $p < 0.001$, but displayed normal extinction in the final FPS test, with no significant increase in startle in the presence of the light, F (1,6) = 2.20, p = 0.18. Figure 6.7. shows a comparison of the startle responding for the subjects on the acquisition FPS Test and on the FPS Test following extinction.

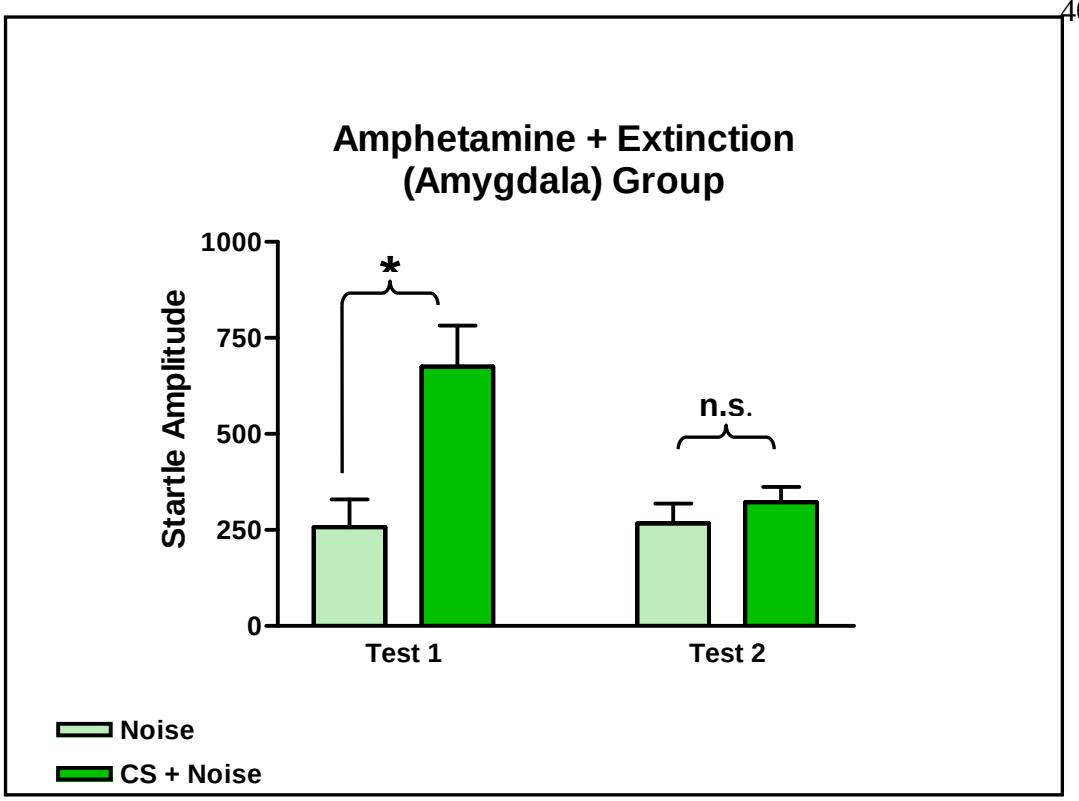


Fig.6.7. **Intra-amygdaloid AMP + Extinction subjects (Experimental 3, *N* = 7). Repeated measures ANOVA for FPS test 1 and 2.**

Intra-amygdaloid subjects showed significant extinction effects and in this respect their responses were distinct from the subjects who received chronic amphetamine infusions. Unlike the intra-VTA subjects there was no significant difference between the responding of the control animals and the amygdala-infused subjects on either the first fear test (F (1, 12) = 2.79, p = 0.12) or the second fear test, F (1, 12) = 0.99, p = 0.34, as shown in Figure 6.8. This suggests that there is a regional dissociation regarding the amphetamine induced impairment of extinction learning, with the extinction impairment appearing to involve the VTA but not the BLA.

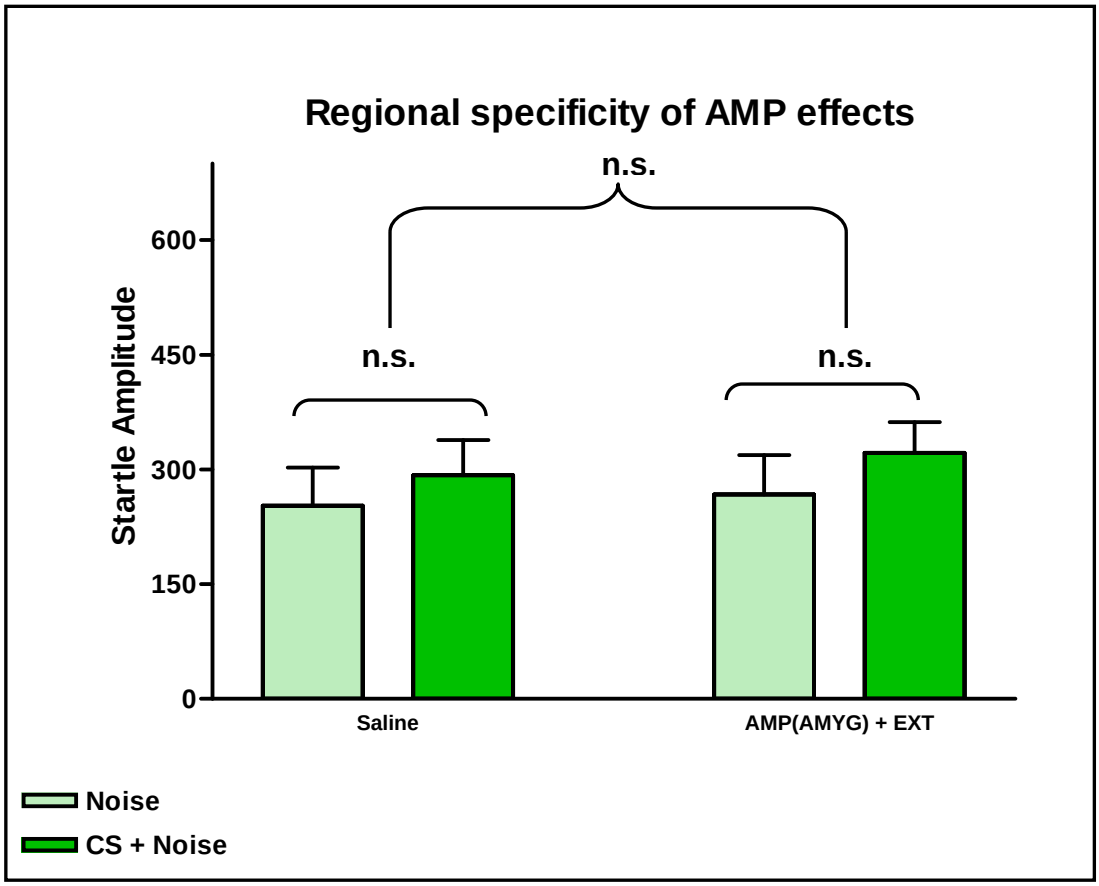


Fig.6.8. Repeated-Measures ANOVA. Between group effects. No significant difference between groups, Exp 3, intra-BLA-AMP + Extinction and Sal + Extinction. p = 0.34, on FPS Test 2.

6. RESULTS - PART B

6. 2. Behavioural Sensitisation

SECTION ONE: Repeated Amphetamine Pre-treatment in VTA vs. Repeated Saline Pre-treatment in VTA.

Repeated intra-cranial amphetamine infusions into the VTA produce long-lasting changes to the sensitivity of that region. A challenge presentation of amphetamine, either peripherally or intra – cranially, following the chronic administration of amphetamine results in heightened behavioural activity in the subject (Kalivas & Weber, 1988, Hooks & Jones, 1992, Cador & Bjijou, 1995). In this study the locomotor effects of a single challenge injection of d-amphetamine (2mg/kg i.p.) were assessed, for subjects who had previously received three infusions intra-cranially, of either d-amphetamine (2.5 µg/side) or saline. Locomotor activity was then recorded at 10 minute intervals for the hour following the i.p. challenge injection of amphetamine.

Extra-cellular concentrations of dopamine and amphetamine have been found to peak in the caudate putamen approximately 30 minutes after peripheral administration of d-amphetamine(Kuczenski, Melega, Cho, & Segal, 1997). Therefore, it was hypothesised that differences between the subjects previously infused with saline in the VTA, and those previously infused with amphetamine, would largely be apparent in the final 30 minutes of testing. As predicted a repeated – measures ANOVA on the final 3 time bins, namely the final 30 minutes yielded a difference between the saline infused subjects and

the amphetamine infused subjects that approached significance, F (2, 34) = 3.17, p= 0.054.

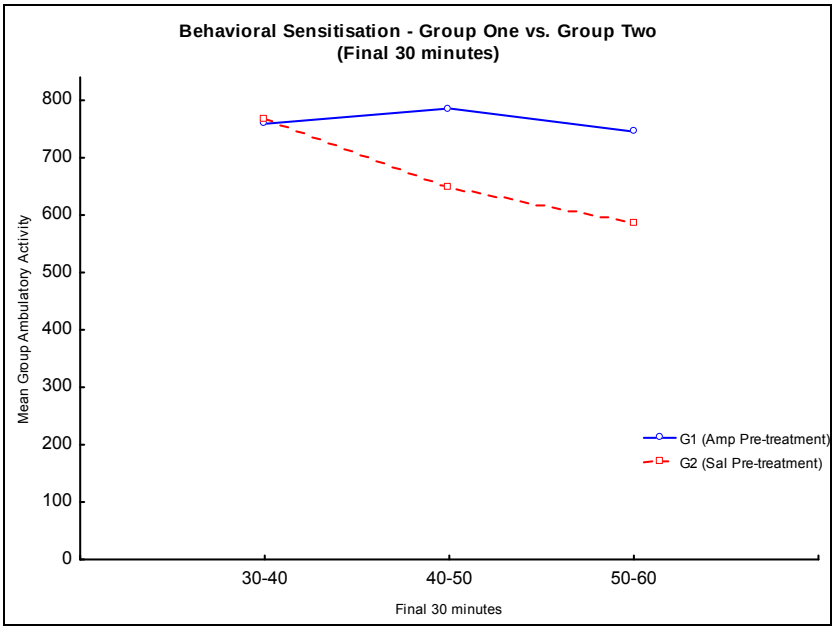


Fig. 4.1. Group Means for final 30 minutes of locomotor activity following challenge injection of d-amphetamine Comparison of pre-treatment effects in VTA subjects.

It is possible that the linear decay of activity levels seen in the Saline Pre-treatment Group, illustrated in Figure 4.1 are consistent with the gradual decline in extra-cellular levels of amphetamine and dopamine as described by Kuczenski and colleagues (1997). Kuczenski and associates(1997) found that after maximal extra-cellular concentrations were reached at 30 minutes a gradual decline in dopamine and amphetamine concentrations occurred with a half life of around 45 minutes. Increases in extra-cellular dopamine and the increases in locomotor activity seen following amphetamine administration share very similar temporal profiles to one another (Hertel et al., 1996).The behavioural responses displayed by the Saline Pre-treatment Group appear to exhibit similar a similar profile, with a behavioural peak at 30 minutes and a gradual decline beginning at 45 minutes. In contrast, the Amphetamine Pre-

treatment Group did not show the gradual decline over the final 30 minutes of the test, rather this group displayed a high level of responding. Our findings are consistent with previous findings of others (Kalivas & Weber, 1988, Hooks & Jones, 1992, Cador & Bjijou, 1995), that repeated administration of amphetamine into the VTA, produces enhanced locomotor activity to a challenge injection. In relation to the extinction deficit seen in the first half of the study, the presence of sensitisation, suggests one possible underlying neural alteration to account for the impairment. It illustrates one important difference between the group that showed the extinction deficit and the group that did not, the group that had the extinction deficit also showed peripheral evidence of a VTA sensitised to the effects of amphetamine.

SECTION TWO: Amphetamine vs. Saline Pre-treatment in the BLA

Three amphetamine infusions into the BLA did not produce any sensitising effects to a subsequent systemic injection of amphetamine. Subjects previously administered amphetamine showed similar behavioural responding to the amphetamine challenge as did those who had previously been infused with saline in the BLA, F (5,45) = 0.81, p = 0.55. Even in the final 30 minutes of recording, where evidence would be expected to be seen, a repeated – measures ANOVA yielded no significant difference between the two pre-treatment groups, F (2, 18) = 0.20, p = 0.82, as shown in Figure 4.2. This lack of behavioural sensitisation seen in the BLA –amphetamine group, further supports the notion that sensitisation of the VTA may be critical for the extinction deficit seen in the VTA – amphetamine group but not seen in the BLA – amphetamine group.

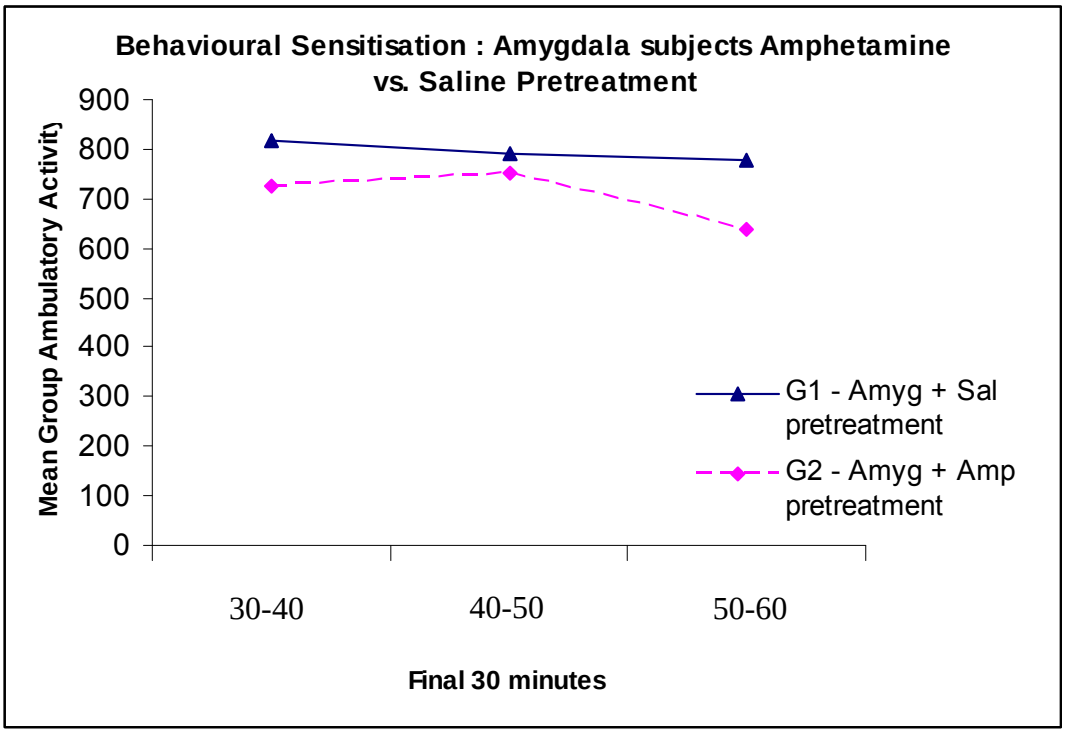


Fig.4.2. Final 30 minutes: Comparison of Pretreatment effects on BLA subjects during behavioural sensitisation test.

SECTION THREE : Effect of Extinction Training on the Expression of Behavioural Sensitisation in the VTA

Like the group of subjects who received repeated amphetamine in the VTA and extinction training, the group that did not receive extinction training showed a different pattern of responding from the saline treated controls in the final 30 minutes, and although not statistically significant, the result approached significance, F (2,30) = 2.83, p = 0.07. There was no overall difference in behavioural responses to the challenge injection, between groups that showed extinction in Part A of the study and groups that did not acquire extinction, F(5,190) = 0.87, p = 0.50. Extinction training itself, did not have any influence on the behavioural responses to the challenge injection, with no significant difference between groups that received extinction training and groups that did not, F (5,190) = 0.67, p = 0.65.

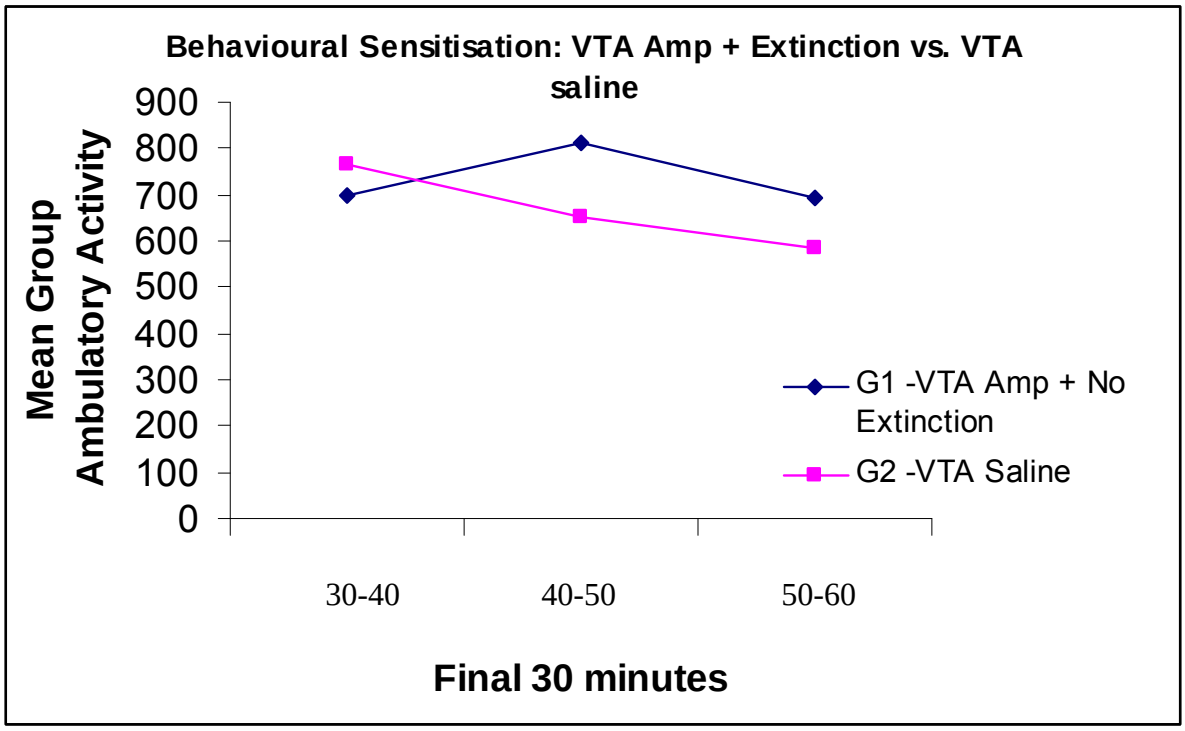


Fig.4.3. Final 30 minutes VTA-Amphetamine + No Extinction Group vs. VTA Saline Controls, F (2,30) = 2.83, p = 0.07.

7 General Discussion

7. 1. Synopsis

Extinction occurs when the continued presentation of the CS, without the corresponding UCS, fails to elicit the CR. In this study the measured CR was the augmentation of the acoustic startle reflex. The augmentation of the startle reflex in the presence of the CS was interpreted as a quantifiable measure of the motivational state of fear. The strength of the fear-potentiated-startle response was taken, relative to the subjects' baseline acoustic startle response, to be indicative of the level of fear elicited by the CS. Extinction training involves the continued unpaired presentation of the CS and is expected to minimise or eliminate the fear-arousing properties of the CS. Subject's who received infusions of d-amphetamine (2.5 µg / 0.5µl) bilaterally into the VTA, prior to the extinction sessions failed to show significant attenuation of their conditioned fear of the light on a subsequent fear test. Although, the fear-potentiated startle exhibited by this group of subjects on the second fear test was of a smaller magnitude than that educed by the first test, nevertheless the fear-potentiated startle exhibited in the presence of the CS was still significantly greater than baseline acoustic startle. These results can be interpreted to suggest that amphetamine reinforced fear responding during CS presentations, this in turn, produced a measurable resistance to extinction.

The possibility that the extinction deficit was produced by a simple enhancement of fear expression as a result of chronic amphetamine infusions

was also addressed by this study. The group of subjects that received chronic amphetamine without extinction, showed significant fear-potentiated startle on both fear tests. If amphetamine was enhancing the expression of fear, it could be reasonably assumed that the fear-potentiated startle exhibited on the second test would be greater than that expressed on the first test. Subject's that received chronic amphetamine infusions did not show greater fear-potentiated startle on the final fear test, indicating that prolonged administration of amphetamine did not significantly alter fear responding to the conditioned stimulus. Interestingly, there was no significant difference between the responses of this group on the final fear test and the responses of the group who received amphetamine and three extinction sessions, further strengthening the suggestion that amphetamine administration is causing an extinction deficit.

As the chronic administration of amphetamine is not enhancing the pre-existing levels of conditioned fear elicited by the CS, amphetamine administration alone is not responsible for the persistent fear seen in subjects who received both amphetamine infusions and extinction sessions. To recapitulate, the only difference between the two groups who received chronic amphetamine treatment was that the first group received three extinction sessions post infusions whereas the second group received no extinction sessions. The outcome was the same for both groups – significant fear on the final fear test – despite the different treatment conditions. As the only manipulated variable between the groups was the administration of extinction sessions to the first group, it would seem reasonable to assume that in some way the administration of amphetamine was impairing the efficacy of the extinction sessions.

The basolateral amygdala is an important region involved in altering the motivational significance held by a particular stimulus, this region has also been implicated in extinction learning (Kellett & Kokkinidis, 2004; Lindgren, Gallagher, & Holland, 2003) In this study, in contrast to the VTA, subject's that received amphetamine infusions prior to each of the three extinction sessions showed normal extinction learning. These results indicate that amygdala dopamine is not important in extinction learning. Although the amygdala is a major recipient of the efferent dopaminergic projections of the VTA, it is not the only region innervated by the VTA. The mesocortical projection from the VTA to the infralimbic cortex may prove an important projection for extinction. This suggestion is based on the findings of Quirk and colleagues (Lebron, Milad, & Quirk, 2004; Santini, Ge, Pena de Oritz, & Quirk, 2004) showing the importance of the medial prefrontal cortex (mPFC) and its' projections to the amygdala in extinction learning. Interestingly, the amygdala is known to modulate the utilization of DA in the mPFC (Goldstein, Rasmusson, Bunney, & Roth, 1996), and it will be interesting to see the developments from future research clarifying the relationship between the amygdala, the VTA and the mPFC during extinction learning.

The second part of this study explored the phenomenon of behavioural sensitisation to amphetamine. Overall subjects pre-treated with three injections of amphetamine (2.5µg/0.5µl/side) into the VTA showed no significant difference in behavioural responding to subjects pre-treated with saline, when given an i.p. challenge of amphetamine (2mg/kg). However, closer examination of the data revealed a difference between the two groups in the final thirty

minutes approached statistical significance. This was consistent with the literature which suggests maximal extra-cellular concentrations of both amphetamine and dopamine peak at 30 minutes after the systemic administration of amphetamine ((Kuczenski, Melega, Cho, & Segal, 1997). Extra-cellular dopamine levels and increases in locomotor activity , share remarkably similar temporal profiles (Hertel et al., 1996). Extra-cellular dopamine and amphetamine have a half-life profile of about 45 minutes (Kuczenski et al., 1997) and the majority of the effects of behavioural sensitisation are seen within the first 50 minutes after the administration of a systemic amphetamine challenge (Kalivas & Weber, 1988). It was therefore anticipated, that the saline pre-treated animals would show a slowing of behavioural responding in the final 30 minutes whereas the amphetamine pre-treated subjects should show a heightened response to the locomotor-activating effects of amphetamine during those final 30 minutes. This hypothesis bore out, with saline pre-treated subjects showing maximal levels of activity at thirty minutes and then gradually waning over the following half hour. By contrast the amphetamine pre-treated subjects showed no such decline over the final 30 minutes and consistently showed higher levels of responding than their saline pre-treated counterparts.

7. 2. Possible Explanations

Is the repeated administration of amphetamine simply causing a learning impairment ?

7. 2. 1 Extinction and the learning of a non-association

In our study we found that subjects who received repeated amphetamine infusions prior to extinction sessions maintained significant fear of the conditioned stimulus on an ensuing FPS test. Expressed in another way those subjects who received chronic amphetamine infusions did not display extinction on the final fear test. The interpretation of the subjects continued fear or non-extinction, depends in part on the chosen definition of extinction. Rescorla (1969) expounds that through learning a stimulus acquires the properties of a conditioned inhibitor, as it comes to elicit the opposite response from the subject as the conditioned excitor. If extinction is viewed as such it is a learned association, i.e. ; the subject learns to replace the conditioned association of CS – UCS, with a new pairing of CS – No UCS. Specifically in our study, extinction learning requires the acquisition of a Light – No shock association.

Traditionally amphetamine has been viewed as a pharmacological facilitator of learning. Systemic administration of methamphetamine prior to discrimination tests – tasks which require the acquisition and application of a designated concept – decreased the time required by subjects to learn an association (Rahmann, 1970). If amphetamine was facilitating the speed at which subjects

learned the new CS – No US association, then the anticipated result would have been a rapidly acquired extinction of the conditioned fear. As the subjects who received amphetamine prior to the extinction sessions did not show enhanced acquisition of the extinction association it appears that the application of intracranial amphetamine is not exerting its' effect by facilitating associative learning. Rather, our results suggest that amphetamine-induced VTA activity is impairing extinction learning. Interestingly, although the BLA has been implicated as a critical region for extinction learning (Kellett & Kokkinidis, 2004) we did not find that dopaminergic manipulation of the BLA resulted in any impairment to the subject's ability to learn an extinction association.

7. 2. 2. Learning conceptualised as synaptic plasticity: Long-term potentiation, learning and memory

Extinction learning can be conceptualised as the acquisition of a new inhibitory association to replace a pre-existing excitatory association. Viewed in this light extinction can also be seen as alterations on a synaptic level to the strength of the initially conditioned association. Long-term potentiation (LTP) is a form of glutamatergic synaptic plasticity, that has been postulated to be the neuronal substrate of learning and memory in fore-brain regions (Jones & Bonci, 2005; Kauer, 2004; Malenka & Nicoll, 1999). LTP is a persistent increase in excitatory synaptic transmission and the opposing process that down-regulates synaptic strength is referred to as long-term depression (LTD) (Bear & Abraham, 1996). The increase in synaptic transmission as a result of LTP, means that the test stimulus involved in the acquisition of LTP, when applied after the induction of

LTP, will elicit a significantly larger response than had previously been evoked. The consistent production of LTP in areas such as the hippocampus, known to be heavily involved in learning and memory, has given support to the proposition that this form of glutamatergic synaptic plasticity could be the neuronal substrate of learning and memory (Malenka & Nicoll, 1999).

7. 2. 3. Biochemistry of Long-term potentiation

The biochemistry of LTP has been extensively studied and although many of the precise mechanisms are still uncertain, there is general consensus about the basic processes involved in the induction of LTP. For review see (Jones & Bonci, 2005; Kauer, 2004; Malenka & Nicoll, 1999; Wolf, Sun, Mangiavacchi, & Chao, 2004). The release of the excitatory neurotransmitter glutamate, during normal synaptic transmission, acts on post-synaptic AMPA and NMDA receptors. The AMPA receptor is permeable to both sodium (Na^+) and potassium (K^+). When the cell is close to its' resting membrane potential, the AMPA receptor provides the majority of the inward current. Under the normal parameters of synaptic transmission the NMDA receptor contributes very little to the inward current received by the post-synaptic cell. This inaction is largely due to the extra-cellular blockade of the NMDA receptor channel by magnesium (Mg^{2+}). The induction of LTP requires the depolarisation of the post-synaptic cell. The depolarisation of the cell causes the Mg^{2+} to dissociate itself from the NMDA receptor channel and allows the flow of Na^+ and Ca^{2+} into the post-synaptic cell. The intracellular rise of Ca^{2+} in the post-synaptic cell is the necessary catalyst for LTP to occur. The generation of LTP is input – specific,

more precisely, the repeated activation of a synapse on a particular cell does not strengthen all synaptic connections on that cell. Although LTP is normally input - specific, strong activation of a synapse can also strengthen adjacent synaptic connections. The associative strengthening of synaptic connections during LTP has been used as a model to explain the neural substrate of classical conditioning. The CS gains associative synaptic strength through its' temporally proximate activation with the UCS.

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7. 2. 4. VTA, Psychomotor Stimulants, LTP and LTD

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7. 2. 5. LTP & LTD

A single systemic injection of cocaine is sufficient to induce LTP in the dopaminergic neurons of the VTA (Ungless, Whistler, Malenka, & Bonci, 2001). Mice injected with a single dose of cocaine, showed a larger AMPA / NMDA receptor ratio than saline injected controls, indicating the occurrence of LTP in cocaine pre-treated controls. Additionally, Ungless and colleagues (2001) found that the single exposure of cocaine was sufficient to induce context-dependent behavioural sensitisation. The behavioural sensitisation was attenuated by the local administration of MK-801 (NMDA receptor antagonist) into the VTA, as was the change in AMPA/NMDA receptor ratio induced by cocaine. The blockade of both behavioural sensitisation and LTP by the administration of NMDA receptor antagonist, MK-801, indicates that both psychomotor induced LTP and behavioural sensitisation are NMDA dependent in the VTA.

The induction of LTP in the VTA appears to be a process common to drugs of abuse. Saal and colleagues (2003) extended the findings of Ungless and co-workers(2001) to include other drugs of abuse such as amphetamine, morphine, ethanol and nicotine. They found that this cohort of drugs all caused the same increase in AMPA/ NMDA ratio at excitatory synapses in midbrain DA cells. Interestingly, the effects of acute stress, using a forced swim task, resulted in an AMPA/NMDA ratio that was even larger than that of the drugs of abuse. Potentially, in this study there could have been a cumulative effect of amphetamine and stress causing a greatly increased ratio of AMPAR/NMDAR at the excitatory synapses in the VTA.

In vitro induction of LTD in the VTA has also been demonstrated (Jones, Kornblum, & Kauer, 2000; Thomas, Malenka, & Bonci, 2000). Unlike LTP in the VTA it is not dependent on the activation of NMDA receptors and is triggered by non-L type voltage-dependent calcium channel activation. Interestingly, VTA slices, bathed in amphetamine, showed inhibition of electrically induced LTD (Jones et al., 2000). The effect of psychomotor stimulants on LTD In vitro, appears to translate to in vivo studies. Ungless and colleagues (2001) observed greater LTD in cocaine pre-treated animals. This finding was assumed to be the consequence of previously elevated synaptic transmission.

7. 2. 6. Possible Explanations arising from LTP/LTD

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7. 2. 7. LTP vs. LTD: Blockade of LTD

LTP and LTD have been proposed to be counter- balancing mechanisms whose net strength governs regional functioning(Bear & Abraham, 1996) . LTP is thought to be the underlying neuronal mechanism responsible for the plasticity involved in responding to new stimuli. Conversely, LTD is the neuronal mechanism involved in losing responsiveness to previously effective stimuli. These two opposing processes appear conceptually to be governing different aspects of learning. LTP seems to be the process necessary to the acquisition of an association, whereas LTD seems to be required for the extinction of a previously acquired association. One way to test the validity of this hypothesis and assess the relative contributions of LTP and LTD to learning is to selectively block one type of plasticity and compare performance on a learning task with untreated controls. Effectively, the study has done just this. Amphetamine administered to the dopaminergic neurons of the VTA is known to block LTD(Jones et al., 2000). The unrestrained contribution of LTP in an inhibitory learning situation, like extinction, may be the cause of the extinction impairment seen in this study.

7.3. Functional consequences of LTP

7. 3. 1. Increased motivational significance of the CS

The functional result of psychomotor stimulant induced LTP in the VTA, is the enhancement of DA transmission to terminal fields (i.e. cortical areas and other limbic structures) (Wolf et al., 2004) . This enhancement of DA transmission, due to LTP, is assumed to persist days after the last administration of the drug. In this state of increased dopaminergic transmission cues associated with the drug attain greater motivational significance(Schultz, 2002). Perhaps, in this study as the CS (light) was continually paired-with the drug state, that the cue later encountered in an LTP altered state had acquired greater motivational (fear-inducing) properties. However, such an explanation rests on the premise that the administration of amphetamine intracranially, had discernable physiological properties for the subject. The literature surrounding the self-administration of psychomotor stimulants to the VTA (Koob & Bloom, 1988) provides support that the intracranial administration of amphetamine to the VTA does have discernable physiologic consequences for the subject.

7. 3. 2. Medial-Pre-Frontal-Cortex and DA transmission

Increased DA transmission in cortical areas, as the result of psychostimulant induced LTP in the VTA raises another possibility MPFC feedback influencing the amygdala. Excitation of MPFC neural activity inhibits amygdaloid functioning and influences extinction (Quirk, Likhtik, Pelletier, & Pare, 2003). MPFC DA

may have an inhibitory influence on MPFC neural activity and this inhibition may underlie the extinction deficit observed in the present study.

7. 4. *Are the neurochemical actions of amphetamine somehow impairing the extinction of conditioned fear?*

7. 4. 1. Effect of amphetamine on dopamine

The acute and immediate effect of systemic amphetamine administration is to increase extracellular dopamine (Bunney & Aghajanian, 1973). Extra-cellular levels of dopamine peak immediately after amphetamine administration but this effect is short-lived, and the overall effect produced by continued amphetamine administration is a decline in basal dopamine levels (Imperato et al., 1996).
The specific mechanisms employed by amphetamine on a cellular level are still relatively uncertain. The increased release of dopamine by amphetamine appears to be caused by the redistribution of dopamine to the cytosol. Leading to a single and significantly increased release of DA from amphetamine treated neurons (Sulzer et al., 1995).The depletion of vesicular stores and the increased initial expulsion of dopamine from the amphetamine treated neuron occurs via reverse transport.

7. 4. 2. DA receptor regulation

AMP iontophoretically administered directly to DA cells causes very little change to the firing rate of the DA neuron itself, the administration of AMP to

postsynaptic neurons in the same region causes a marked depression of firing rates(Aghajanian & Bunney, 1973). It has been suggested that a feedback pathway is mediating the depressant effects of AMP on DA neurons, rather than a direct post-synaptic action of the drug (Bunney & Aghajanian, 1973).

More recently, the psychomotor stimulant has been found to affect the dopaminergic neurons of the CNS in both an excitatory and inhibitory manner (Shi, Pun, Zhang, Jones, & Bunney, 2000). Under basal conditions dopamine release is primarily inhibited via the local D_2 receptor. Under conditions where the extracellular level of dopamine has been significantly raised, such as following the application of AMP, the concurrent activation of both D_1 and D_2 receptors is necessary to inhibit the DA neuron (Adell & Artigas, 2004; Shi, 2000).

Interestingly, the successful blockade of the inhibitory effects of the D2 receptor with raclopride, following the administration of amphetamine, led to the discovery that the excitatory effects of AMP administration are non-DA mediated (Shi et al., 2000). Amphetamine therefore appears to have dual effects on dopamine cells, namely DA mediated inhibition and non-DA mediated excitation.

7. 4 . 3. Differences between somatodendritic and terminal release of DA

The administration of amphetamine causes the release of DA in the midbrain region, consequently, DA is released from not only the nerve terminals, but also from the cell body and its proximal dendrites(Cragg & Greenfield, 1997; Geffen, 1976; Korf, Zieleman, & Wseterink, 1976). The subsequent exodus of DA from the neuron activates both long-loop feed back mechanisms and autoreceptors which combine to inhibit the firing of the neuron(Bunney, Walters, Roth, & Aghajanian, 1973; Shi, 2000). Both the release and feedback inhibition of DA differ between somatodendritic and axon terminal regions. Quantitatively, DA released from the axon terminals is consistently greater than the that released in the somatodendritic regions(Cragg & Greenfield, 1997). During normal DA function, uptake by the DA transporter (DAT) is less efficient in somatodendritic areas than in terminal regions (Adell & Artigas, 2004; Cragg, Rice, & Greenfield, 1997; Geffen, 1976). This function is consistent with the larger number of DA uptake sites in terminal areas. As a result of the varying degrees of uptake efficiency a different time course of action exists for somatodendritic DA release and terminal DA release. The differences between the somatodendritic release of DA and the axon terminal release of dopamine also include variation in autoreceptor regulation (Cragg & Greenfield, 1997). The differences in the regulation of extracellular dopamine appear to be dependent upon location, autoreceptor regulation and the presence of DAT. Approximately, 95% of intracellular DAT in VTA dopaminergic neurons is located within the perikaya and it’s immediate dendrites. The remaining 5% of

DAT is found within the unmyelinated axons of the cell(Nirenberg et al., 1997). Potentially the disparate results seen in the non-extinction of subjects who received amphetamine chronically into the VTA, and the extinction of those subjects who received amphetamine into the BLA, could be due to the underlying differences in the regulation of extracellular dopamine in the two different regions. The discrepancy could be explained as a difference in the regulation of extracellular dopamine in terminal regions compared to somatodendritic regions.

7. 4 . 4. Dopamine release and neurochemical control

The firing rate of the DA neuron can act as an error signal for the prediction of reinforcement. When an anticipated event does not occur, dopamine firing rates decrease. Dopamine therefore is signalling the quality of the environment as a predictor of the anticipated event. For review (Schultz, 2002). During extinction sessions in normal circumstances, dopamine would act as an error signal. The firing rates of the dopamine cell would be expected to decrease as the CS (light) becomes a consistently poorer predictor of the shock (UCS). The administration of amphetamine immediately prior to extinction sessions will cause an immediate increase in extra-cellular dopamine, followed by inhibition of the firing rate of the dopamine neuron. The pharmacological manipulation used in this study may have affected the ability of dopamine to function as an effective error signal. The resultant decrease in dopamine firing rates that would under normal circumstances accompany the weakening of the CS as a predictor of the UCS, had already been pharmacologically instigated prior to the presentation of the unpaired UCS. Conversely, the immediate increase in extra-

cellular dopamine post infusion may mean an overly active error signal. However, if the pharmacological interference is creating an error signalling deficit, then the extinction deficit should have been seen in both the group receiving infusions into the amygdala and the subjects receiving infusions into the VTA. As the two groups did not show identical results, it suggests that the underlying mechanism is more complex than a deficit in neurochemical error signalling.

7. 5. *Is the extinction impairment the result of an amphetamine effect on memory?*

Historically, the systemic administration of amphetamine has been linked to improvements in memory – related tasks(Carr & White, 1984; Doty & Doty, 1966; Rahmann, 1970). The enhancement produced by amphetamine on memory-related tasks is both dependent upon dose and temporal proximity. Low doses of methamphetamine prior to testing were found to decrease subject learning latency in discrimination tasks and also significantly prolonged subject retention (long-term memory) of the correct responses to these tasks (Rahmann, 1970). Amphetamine administered post training also facilitates the consolidation of memory. This is a temporally constrained effect with facilitation found when amphetamine is administered at proximal but not distal intervals following training (Doty & Doty, 1966).

Carr and colleagues (1984) found that a post-training injection, either systemically of intracranially facilitated the consolidation of recently formed

associations. Additionally they found a significant correlation between stereotypic behaviour and memory retention i.e. subjects who showed the greatest amount of stereotyped behaviours following the administration of amphetamine also showed the strongest conditioned suppression when tested 48 hours later. The enhancement of memory seen here was retroactive, insofar as the subjects received amphetamine immediately after to the conditioned suppression training. Those who received amphetamine immediately after the training showed greater suppression than those who received delayed administration of amphetamine, indicating a transient window for facilitation of a previously learned association.

Interestingly, the neurotransmitter NMDA also seems to exert a transient effect on memory consolidation (Santini, Muller, & Quirk, 2001). Systemic administration of NMDA receptor blocker CPP prior to extinction learning did not interfere with acquisition of extinction 1 hour after extinction nor did it at 48hours, however an extinction impairment was displayed by subjects 24hours after extinction learning. This study highlights the importance of considering the temporal elements involved in the consolidation of extinction learning, especially when the pharmacologically manipulating extinction learning. The extinction impairment seen in this study may have been due to some interaction between the temporal aspects of extinction consolidation and amphetamine itself.

Extinction of a response is not due to the ‘forgetting’ of the conditioned association over the course of time. Fear related behaviours present in a conditioned – fear paradigm, will persist across large time periods (McAllister,

McAllister, Scoles, & Hampton, 1986) and also show considerable tenacity as exemplified by phenomena such as spontaneous recovery and renewal. Given the longevity of the conditioned fear association and the transient nature of neurochemical correlates with the facilitation of previously learned associations, the administration of amphetamine immediately prior to extinction sessions, may have been retroactively enhancing the CS-UCS association learned in that context.

7. 6. Behavioural Sensitisation

Primarily the in vivo, intracranial induction of amphetamine sensitisation has focused on the dopaminergic projection from the VTA to the nucleus accumbens (mesoaccumbens pathway), a pathway largely involved in the acute locomotor effects of amphetamine administration (Cador, Bjijou, & Stinus, 1995; Hooks, Jones, Liem, & Justice, 1992; Perugini & Vezina, 1994; Vezina, 1993; Vezina & Stewart, 1990). Rats who received three infusions of amphetamine (3µg/0.5µl) into the nucleus accumbens (NACC) did not show significant augmentation of behavioural responding to a challenge i.p. injection of amphetamine (1mg/kg) compared to control subjects. This regimen acutely produced a significant increase in ambulatory activity, but chronically did not induce behavioural sensitisation (Hooks et al., 1992) in the NACC. Conversely, the same chronic treatment in the VTA induced behavioural sensitisation to a challenge injection of amphetamine (Hooks et al., 1992; Kalivas & Weber, 1988; Vezina & Stewart, 1990), while an acute infusion of amphetamine into the VTA did not increase locomotor activity in subjects (Kalivas & Weber, 1988).

These findings emphasise the distinct contribution of each neural region to the phenomenon of amphetamine-induced behavioural sensitisation.

Intracranial induction of behavioural sensitisation is not only region specific, but is also dose dependent. A foundational study by Kalivas and Weber (1988) showed that two infusions of 1.5 µg/µl amphetamine intra-VTA were sufficient to produce significantly enhanced locomotor effects to an i.p. challenge injection (1.0mg/kg). While others have failed to achieve sensitisation at such low doses(Cador et al., 1995), the most consistently used dose is 2.5 µg/µl(Cador et al., 1995; Perugini & Vezina, 1994; Vezina, 1993; Vezina & Stewart, 1990). Subsequent studies found that the intra-VTA injections of amphetamine cross-sensitised subjects to other drugs of abuse such as morphine (Vezina & Stewart, 1990), and cocaine (Hooks et al., 1992). Additionally, behavioural sensitization can also be elicited by an intra-cranial challenge injection (Cador et al., 1995; Perugini & Vezina, 1994) of amphetamine to the nucleus accumbens. Controversy, remains about the generalisabilty of the intracranial induction of behavioural sensitisation via amphetamine to the entire class of psychostimulants. Four infusions of cocaine into the VTA, at a variety of dosages did not produce enhanced locomotor activity to systemic challenge of cocaine. This result could not be ascribed to the anaesthetic effects of cocaine as WIN 35, 065-2 (cocaine analogue without anaesthetic properties) did not produce sensitization either (Steketee, 1998). However, Cornish and Kalivas(2001) were subsequently able to produce behavioural sensitisation with cocaine using identical procedures to Steketee(1998)

The subjects in this study, consistent with the literature did show behavioural sensitisation to the amphetamine challenge, following repeated intra-VTA amphetamine (2.5 µg/0.5µl). This effect within the context of this study primarily helped to confirm that the subjects had in fact been successfully receiving amphetamine to the VTA during the fear extinction trials. Additionally, the extinction impairment seen in the behaviourally sensitised subjects allows the possibility that the neurochemical basis of one could underlie the other. More specifically it raises the possibility that the neurochemical basis of the extinction impairment is a sensitised mid-brain dopaminergic projection.

7. 7. Possible Confounds

7. 7. 1. The problem of state dependency

Fear extinguished in a drug state, may be renewed when the subject is placed back in the context, in a non-drug state and tested (Bouton, 1993). This tenet, known as state - dependency , is a potentially confounding factor in a study of this nature. Bouton and colleagues (1990) found state – dependent retention of extinction in subjects treated with benzodiazepines. The subjects displayed successful acquisition of extinction whilst under the influence of the drugs, but failed to show extinction when tested in the non-drug state. This state – dependent retention of extinction has been shown with a variety of drugs including pentobarbital, amobarbital and alcohol(Barry, Etheredge, & Miller, 1965; Cunningham, 1979; Overton, 1964). With regard to this study, the

possibility that the extinction impairment is due to a failed transfer of learning from the drug state to the non-drug state, is a potential confound. State-dependency in this paradigm necessarily rests on the assumption, that extinction was acquired in the drug state and subsequently forgotten in the non-drug state. The group that received amphetamine infusions into the amygdala showed normal extinction learning. This result throws into doubt any question of state dependency, as the amygdala subjects successfully transferred extinction learning from the drug state to the final fear test, which was in a non-drug context.

7. 7. 2. Introduction of a novel stimulus

Another possibly confounding factor that would benefit from further explanation is whether the introduction of the novel amphetamine prior to extinction produces an extinction deficit simply because it acts as a novel disruptor. This presupposes that the intra-cranial administration of amphetamine is a discernable physiologic to the subject. Intra-cranial self-administration studies support the idea that the intra-cranial infusion is a discernable physiologic event to the subject. Animals will self administer a large variety of drugs of abuse directly to the VTA, including morphine, ethanol (for review see(McBride, Murphy, & Ikemoto, 1999), suggesting that the effects of drugs administered intra-cranially are discernable to the subject. A potential way that this problem, of the introduction of a novel stimulus, could be countered would be to have a group who received amphetamine infusions prior fear conditioning, to determine whether the introduction of amphetamine interfered with the acquisition of an association. However, once again the fact that the group who received

amphetamine infusions into the basolateral amygdala prior to extinction showed the successful acquisition of extinction, brings into to question the necessity of repudiating the novel-disruptor problem.

7. 8. Discussion and Future Extensions

The results of this study have interesting implications for our understanding of fear, psychosis and schizophrenia in general. As normal fear is characterised by an adaptive response to an aversive or dangerous situation, abnormal fear can be characterised by defensive behavioural response in the absence of an immediate or future threat. Persistent fear of an innocuous stimulus can lead to the development of phobias and paranoid psychoses. As, such this type of fear can be conceptualised as an extinction impairment on the part of the individual. Whether this impairment is the result of a neurochemical imbalance or the consequence of a learning deficit, namely the inability of the subject to acquire extinction associations, it is of importance to understand the processes underlying this persistent fear, to better develop ways of treating it.

Subjects who received repeated infusions of amphetamine into the VTA prior to extinction sessions showed an extinction impairment on a subsequent fear test. Subjects who showed this extinction impairment also exhibited behavioural sensitisation to a subsequent systemic injection of amphetamine. The behavioural sensitisation seen in these subjects provides a possible candidate for the underlying neural change involved in the extinction impairment. This is consistent with the fact that drugs which facilitate the action of dopamine, like amphetamine, are able to induce the positive psychotic symptoms of

schizophrenia(Angrist, Sathananthan, Wilk & Gershon, 1974). Among the positive symptoms of schizophrenia are delusional thoughts, phobias and paranoid ideations. Neurochemical sensitisation of the mesolimbic dopaminergic system has been implicated as a contributing factor in the development of schizophrenia (Glenthoj & Hemmingsen, 1997, Lieberman, Sheitman & Kinon, 1997, Laurelle, 2000). The persistent fear seen in our subjects following the application of a dopaminergic agonist, amphetamine, could potentially be the result of a sensitised mesolimbic projection in these subjects.

The extinction impairment following the application of amphetamine prior to extinction sessions, was not due to an increase in the expression of conditioned fear in subjects. This allows the possibility that the extinction impairment seen in this study was due to an effect of amphetamine on either extinction learning or the consolidation of extinction memory. As previously outlined amphetamine administration to the dopaminergic neurons of the VTA, has been shown to precipitate synaptic changes both inducing LTP (Saal et al., 2003) and blocking the induction of LTD (Jones et al., 2000). A pertinent question about synaptic alterations arises in relation to amphetamine, does the block of LTD by dopamine releasing drugs does in fact promote LTP? (Kauer, 2004) If so, what effect might this be having on the subjects ability to lose responsiveness to a previously relevant stimuli? To further clarify the extinction impairment found in our study an investigation into the synaptic changes occurring in vitro, following the repeated application of amphetamine, would nicely complement the findings of this study.

Additionally, the disparity in findings between the BLA and the VTA will need to be investigated more thoroughly to firmly establish whether this extinction impairment is truly a regionally specific one. This could have important ramifications for our understanding of the neural regions critical for extinction learning. One way to achieve this would be to manipulate other terminal regions of the meso-cortico-limbic pathway to more precisely establish the bounds of regional involvement. In addition, it would be interesting to replicate this study with a variety of other dopaminergic agents to more precisely establish the role of DA neurons in this extinction impairment. These possibilities alone or in conjunction provide a large variety of potential avenues to explore to better understand the extinction deficit found following the administration of amphetamine to the VTA prior to extinction sessions.

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